REMARKS

Amendments to the Claims

Claims 2, 6 and 13 have been canceled. Claims 1, 3-5, 7-8, 11-12 and 14-15 have been amended. New Claims 16-25 have been added.

Claims 1, 3-5 and 7-8 have been amended to clarify that the pathology treated is "TNFα-mediated hepatitis." Support for these amendments is found in the specification, for example, at page 16, lines 15-19 and page 57 line 17 to page 59, line 14.

Claims 11, 12, and 14-15 have been amended to clarify that the inflammation treated is associated with "TNFα-mediated hepatitis." Support for these amendments is found in the specification, for example, at page 16, lines 15-19 and page 57, line 17 to page 59, line 14.

Claims 1, 5 and 12 have been further amended to recite that the administered antibody competitively inhibits binding of "human TNFα" to "anti-TNFα chimeric" monoclonal antibody cA2. Support for these amendments is found in the specification, for example, at page 10, lines 8-15 and page 19, lines 7-24.

Claim 1, 5, 7-8, 11-12 and 15 have been further amended to recite that the antibody administered is a "TNFα-inhibiting amount of an anti-TNFα chimeric antibody." Claims 3 and 14 have been further amended to recite that the antibody administered is a "TNFα-inhibiting amount of anti-TNFα chimeric monoclonal antibody cA2." Support for these amendments is found in the specification, for example, at page 10, lines 8-15 and page 19, lines 7-24.

Claim 4 has been further amended to recite "...administering to the human at least one anti-TNFα chimeric monoclonal antibody cA2, or an antigen-binding fragment thereof." Support for the amendment to Claim 4 is found in the specification, for example, at page 10, lines 8-15 and page 19, lines 17-24.

New Claim 16 is directed to the method of Claim 1 wherein said chimeric anti-TNFα antibody binds to a neutralizing epitope of human TNFα. Support for New Claim 16 is found in the specification, for example, at page 10, lines 8-15 and page 19, lines 7-16.

New Claim 17 is directed to a method of treating TNFα-mediated hepatitis in a human comprising administering to the human an effective TNFα-inhibiting amount of an anti-TNFα antibody, wherein said anti-TNFα antibody competitively inhibits binding of human TNFα to chimeric anti-TNFα monoclonal antibody cA2. Support for New Claim 17 is found in the

specification, for example, at page 10, lines 8-15; page 19, lines 7-16; and page 57 line 17 to page 59, line14.

New Claim 18 is directed to the method of Claim 1 wherein said anti-TNFα antibody binds with high affinity to a neutralizing epitope of human TNFα. Support for New Claim 19 is found in the specification, for example, at page 19, lines 7-24.

New Claim 19 is directed to the method of Claim 1 wherein said anti-TNFα antibody binds to a neutralizing epitope of human TNFα *in vivo* with an affinity of at least 1 x 10^8 liter/mole, measured as an association constant (Ka), as determined by Scatchard analysis. Support for New Claim 19 is found in the specification, for example, at page 10, lines 8-15, and Example X, particularly page 80, line 24 to page 81, line 12.

New Claim 20 is directed to the method of Claim 1 wherein said anti-TNFα antibody is administered to the human by means of parenteral administration. New Claim 21 is directed to the method of Claim 1 wherein said anti-TNFα antibody is administered to the human by means of intravenous administration. New Claim 22 is directed to the method of Claim 1 wherein said anti-TNFα antibody is administered to the human orally. Support for New Claims 20-22 is found in the specification, for example, at page 59, lines 23-29.

New Claim 23 is directed to the method of Claim 1 wherein said TNFα-inhibiting amount of the anti-TNFα antibody comprises a single or divided dose of about 0.1 - 50 mg/kg. New Claim 24 is directed to the method of Claim 18 wherein the single or divided dose is selected from the group consisting of: about a 0.1 - 1 mg/kg dose, about a 1.0 - 5 mg/kg dose, about a 5 - 10 mg/kg dose and about a 10 - 20 mg/kg dose. Support for New Claims 23 and 24 is found in the specification, for example, at page 60, lines 7-24.

New Claim 25 is directed to the method of Claim 1 further comprising administering to the human an effective amount of a therapeutic agent selected from the group consisting of: radiotherapeutics, cytotoxic drugs, monoclonal antibodies, chimeric antibodies, antibody fragments, antibody regions, lymphokines, cytokines, hemopoietic growth factors and immunoglobulins. Support for New Claim 25 is found in the specification, for example, at page 62, lines 4-23 and page 63, lines 3-7.

No new matter has been added by the amendments. Therefore, entry of the amendments into the application is respectfully requested.

Correspondence Address

Please note that the undersigned Attorney has taken over responsibility for this application. A Notice of Change of Contact Attorney is being filed herewith.

Sequence Listing

Applicants thank the Examiner for noting that the instant application is in sequence compliance for patent applications containing nucleotide sequence and/or amino acid sequence disclosures.

Amendments to the Specification

The Examiner states that the application is to be reviewed and all spelling, trademarks, and like errors corrected, and that the first line of the specification should be amended to update the status of the priority documents.

Applicants have amended the specification to comply with the requirement to indicate trademarks, to correct typographical errors and to update the status of a related application. Support for these amendments is found throughout the specification. In addition, Applicants have amended the paragraph at page 58, line 1 through page 59, line 14 to recite "hepatitis, e.g., alcohol-induced hepatitis." Support for this amendment is found in the title; originally-filed Claims 1-8 and 11-15; and page 59, line 11. No new matter has been added by the amendments. Therefore, entry of the amendments into the application is respectfully requested.

Priority

The Examiner states that "[t]he filing date of the instant claims is deemed to be the filing date of the instant application USSN 10/043,436, filed 1/10/02." The Examiner further states that:

> It does not appear [that] the priority applications provide sufficient written description for treating hepatitis with cA2-specific antibodies. For example, it appears that the disclosure of "hepatitis pathologies" is limited to the instant claims only. Page 59, Section (F) of the instant specification discloses "(F)

> alcohol-induced hepatitis" and not the more generic recitation of "hepatitis pathologies" recited in the instant claims. It appears that the disclosure of the priority applications is limited to "(F) alcohol-induced hepatitis" and not the more generic recitation of "hepatitis pathologies" recited in the instant claims.

Applicants respectfully disagree. The instant claims are entitled to claim the benefit of priority application USSN 07/670,827 (filed March 18, 1991). Priority application USSN 07/670,827 provides sufficient written description and enablement for treating TNFα-mediated human disease, including hepatitis. USSN 07/670,827 discloses that the "[h]igh affinity chimeric anti-TNFα mAbs of the present invention, which have potent TNFα neutralizing activity, including TNFα-neutralizing fragments thereof, are useful as therapeutic agents for TNFα-mediated human disease...." (page 10, line 22-25) This priority application teaches and enables treatment of a representative number of species of the genus of "TNFα-mediated diseases," including "rheumatoid arthritis," "Crohn's disease," "sarcoidosis," "inflammatory diseases" and "alcohol-induced hepatitis" with the claimed antibodies. (See USSN 07/670,827 at page 39, line 20 to page 40, line 9 and page 10, lines 22 to page 11, line 4)

Hepatitis is a TNFα-mediated disease. Hepatitis is defined as an inflammation of the liver from any cause. (See The Merck Manual of Medical Information, Berkow *et al.*, Pocket Books, page 571-574 (1997) (Exhibit A)) Although the specification does not provide a specific example directed to TNFα-mediated hepatitis, the mechanism of treatment would be the same regardless of the TNFα-mediated disease. Furthermore, the Examiner indicates that treatment of alcohol-induced hepatitis is enabled by the current specification. Treatment of hepatitis would be the same regardless of its cause.

Therefore, the priority application 07/670,827 (filed March 18, 1991) provides sufficient written description and enablement for treating TNFα-mediated hepatitis, and Applicants are entitled to claim the benefit of its filing date. This priority application has been properly referenced on page 1 of the specification in compliance with 35 U.S.C. § 120.

Further, at the very least, Applicants are entitled to priority to February 4, 1994. Applicants note that the Examiner has cited Applicants' own priority patent (Le *et al.* U.S. Patent No. 5,919,452) as prior art. The Examiner states in the 35 U.S.C. § 102 (b) rejection that "[t]he claimed functional limitations would be inherent properties of the referenced methods [taught in U.S. Patent No. 5,919,452] to treat alcohol-induced hepatitis with recombinant cA2-specific

antibodies." (Office Action at page 6) Le *et al.* (5,919,452) was filed February 4, 1994 and published July 6, 1999 and it also claims the benefit of priority to the same U.S. priority application (U.S. Serial No. 07/670,827) as the subject application. As discussed below, in order to qualify as an anticipatory reference, a reference must meet the requirement of enablement. Therefore, if Applicants' disclosure in U.S. Patent No. 5,919,452 is sufficient to qualify as prior art, then U.S. Patent No. 5,919,452 is sufficient to support the claims in the subject application, and the claims, at the very least, are entitled to the benefit of priority to the filing date of February 4, 1994.

Moreover, priority application USSN 07/943,852, filed September 11, 1992, provides additional support for the claimed methods of treating TNFα-mediated diseases. For instance, Example XIX discloses the clinical effectiveness of treating a known TNFα-mediated disease, rheumatoid arthritis, by administering the recited anti-TNFα antibodies. Although there is not a specific example directed to TNFα-mediated hepatitis, the mechanism of treatment would be the same regardless of the TNFα-mediated disease. This disclosure provides even further support for the claimed treatment methods. This priority patent has been properly referenced on page 1 of the specification in compliance with 35 U.S.C. § 120.

Objection to the Specification Under 37 C.F.R. § 1.75(d) and M.P.E.P. § 608.01(l)

The Examiner states that "[t]he specification is objected to as failing to provide proper antecedent basis for the claimed subject matter. See 37 C.F.R. 1.75 § (d)(1) and M.P.E.P. § 608.01(l). The Examiner further states that:

> It appears that the disclosure of "hepatitis pathologies" is limited to the instant claims only. Page 59, Section (F) of the instant specification discloses "(F) alcohol-induced hepatitis" and not the more generic recitation of 'hepatitis pathologies" recited in the instant claims. Applicant is required to amend the specification to provide proper antecedent basis for the claimed recitation of hepatitis pathologies".

Applicants have amended the specification at page 58, line 1 to page 59, line 14 to provide further literal support for "hepatitis," thereby obviating the rejection. (37 C.F.R. § 1.75(d) and M.P.E.P. § 608.01(l)) Moreover, the claims have been amended to recite "hepatitis." Reconsideration and withdrawal of the objection are respectfully requested.

Rejection to Claims 1, 3-5, 11-12 and 14-15 Under 35 U.S.C. § 112, first paragraph

Claims 1, 3-5, 11-12 and 14-15 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it most nearly connected, to make and/or use the invention. The Examiner further states that:

> It is apparent that the cA2 antibody is required to practice the claimed invention. As a required element, it must be known and readily available to the public or obtainable by a repeatable method set forth in the specification. If it is not so obtainable or available, the enablement requirements of 35 USC 112, first paragraph, may be satisfied by a deposit of the cell line/hybridoma which produces this antibody. See 37 CFR 1.801-1.809.

Applicants respectfully disagree. The cA2 antibodies can be obtained from publicly available material with only routine experimentation. Therefore, the biological materials for cA2 antibodies need not be, and have not been, publicly deposited.

Applicants direct the Examiner's attention to the Federal Circuit decision in *In re Wands*, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988) (a copy of which is attached as Exhibit B for the Examiner's convenience). The claims at issue in *In re Wands* recited methods for an immunoassay using high affinity monoclonal antibodies that the Appellants found to have unexpectedly high sensitivity and specificity. The position of the PTO was that the data showed that the production of the antibodies is unpredictable and unreliable, so that it would require undue experimentation for one skilled in the art to make them. However, the court in *In re Wands* disagreed, noting that "[e]nablement is not precluded by the necessity for some experimentation such as routine screening," as long as the experimentation was not undue. *Id.* at 1404. The court concluded that undue experimentation would not be required to practice the claimed invention.

The court first stated that "Wands' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples." *Id.* at 1406. The court further stated that "[t]here was a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known." *Id.* The court in *In re Wands* recognized that the nature of monoclonal antibody technology is

such that it involves screening hybridomas to determine which ones secrete antibodies with desired characteristics, and that practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody. *Id.* The court went on to state that "in the monoclonal antibody art it appears that an 'experiment' is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen." *Id.* at 1407.

Chimeric A2 (cA2) is a monoclonal anti-TNFα antibody consists of the antigen binding variable region of the high-affinity neutralizing mouse antihuman TNF IgGl antibody, designated A2, and the constant regions of a human IgGl, kappa immunoglobulin. The human IgGl Fc region improves allogeneic antibody effector function, increases the circulating serum half-life, and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric A2 is derived from the variable regions of the murine A2. Chimeric A2 neutralizes the cytotoxic effect of both natural and recombinant human TNF. (See, for example, instant Detailed Description at page 34, line 10 to page 35, line 4). Examples I-IX teach the production, characterization and expression of the cA2 antibody. Examples X-XII teach assays for screening the cA2 antibody.

In considering the factors enumerated in *In re Wands*, Applicants' disclosure provides considerable direction and guidance on how to practice their invention, and presents numerous working examples. For example, the sequences of the variable regions of the antibodies are disclosed in Figures 16A-16B. In addition, the specification teaches methods of producing the claimed cA2 antibodies according to the present invention (See instant Detailed Description at page 32, lines 7 through 24; page 34, line 10 through page 35, line 4; and Examples III-IX).

Additionally, Applicants' disclosure teaches methods of cloning a polynucleotide encoding an anti-TNF variable or constant regions. (See, for example, instant Detailed Description at page 28, line 3 through page 31, line 2). Furthermore, it teaches that preferred anti-TNF monoclonal antibodies include those which will competitively inhibit *in vivo* the binding to human TNFα of anti-TNFα murine monoclonal antibody A2, chimeric monoclonal antibody cA2, or an antibody having substantially the same specific binding characteristics, as well as fragments and regions thereof. (See, for example, page 19, lines 17-20). It also teaches preferred methods for determining monoclonal antibody specificity and affinity (See, for

example, instant Specification at page 19, line 25 through page 20, line 2, and Examples X and XI). In addition, there was a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known.

Thus, a person of skill in the art would not be subject to undue experimentation without a reasonable expectation of success in order to make and screen cA2 antibodies which would have these claimed elements.

A deposit is not required because the disclosure is sufficient to enable production of the claimed antibodies. No more is required. The Examiner has failed to present any evidence which suggests that anti-TNFα antibodies with the claimed specificity are unusually difficult to isolate.

In addition, Applicants' written specification fully enables the practice of the claimed invention because the claimed cA2 antibodies can be made from readily available starting materials using methods that are well known in the art and taught in detail in the specification. As discussed above, and as detailed in the specification, cA2 is derived from the A2 antibody. The A2 antibody was publicly available at least as of April 19, 1992. (See Declaration of Jan Vilcek M.D., hereinafter "Vilcek Declaration" at ¶ 5)

Furthermore, as noted by the Examiner, the claims encompassing the cA2 antibody issued in the related priority patent, U.S. patent No. 5,919,452. As is clear from the prosecution history, no deposit was necessary to satisfy the enablement requirement. Moreover, Applicants' argument that the claims are enabled and a cA2 deposit is not required has also been found persuasive in other related U.S. Applications, including USSN 09/756,301, now U.S. Patent No. 6,790,444.

As discussed above, the instant Specification and figures, together with what was known and available in the art, provide ample teachings such that one of skill in the art would not be subject to undue experimentation in order to make or use the claimed antibodies. Thus, the skilled artisan is enabled to make and use the claimed invention commensurate in scope with the claims. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection to Claims 1-15 Under 35 U.S.C. § 112, first paragraph

The Examiner has rejected Claims 1-15 under 35 U.S.C. § 112, first paragraph, "because the specification, while being enabling for the 'TNF-α specificity'; does not reasonably provide

enablement for any 'TNF-specificity' having such specificities."

Applicants respectfully disagree. As noted above, Applicants have canceled Claims 2, 6, and 13. Further, to expedite prosecution, Applicants have amended Claims 1, 3-5, 7-8, 11-12, and 14-15 to recite that the claimed antibodies are anti-TNFα antibodies. Applicants have exemplified that the cA2 antibody competitively inhibits and binds with high affinity a neutralizing epitope of human TNF-α. Therefore, particularly as amended, the claims are enabled.

However, it should also be noted that anti-TNFα antibodies are not the only antibodies supported by the specification. As indicated in the specification, the present invention provides anti-TNF compounds and compositions comprising anti-TNF antibodies (Abs) and/or anti-TNF peptides which inhibit and/or neutralize TNF biological activity *in vitro, in situ* and/or *in vivo*, as specific for association with neutralizing epitopes of human tumor necrosis factor-alpha (hTNFα) and/or human tumor necrosis factor β (hTNFβ). (Page 16, lines 15-19.) Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection to Claims 1-15 Under 35 U.S.C. § 112, first paragraph

The Examiner has rejected Claims 1-15 under 35 U.S.C. § 112, first paragraph, "because the specification, while being enabling for treating "alcohol-induced hepatitis", does not reasonably provide enablement for any "hepatitis pathology." The Examiner further states that:

> It appears that the disclosure of "hepatitis pathologies" is limited to the instant claims only. Page 59, Section (F) of the instant specification discloses "(F) alcohol-induced hepatitis" and not the more generic recitation of "hepatitis pathologies" recited in the instant claims. There is insufficient guidance and direction as to the nature or the targeted endpoints of "hepatitis pathologies" other than treating "alcohol-induced hepatitis".

As discussed above, Claims 1, 3-5, 7-8, 11-12 and 14-15 have been amended to recite "TNFα-mediated hepatitis. Claims 9 and 10 are dependent on Claim 1 and, therefore, contain the same element. Claims 2, 6 and 13 have been canceled. In addition, Applicants have amended the specification at page 58, line 1 to page 59, line 14 to further recite "hepatitis." *See* 37 C.F.R. § 1.75(d) and M.P.E.P. § 608.01(l)). Therefore, the disclosure of "hepatitis" is not

limited to the instant claims only.

Further, Applicants note that the Examiner has cited as prior art one of Applicants' priority patents (U.S. Patent No. 5,919,452), which has substantially the same specification. As discussed below, in order to qualify as an anticipatory reference, a reference must meet the requirement of enablement. Therefore, if Applicants' disclosure regarding hepatitis in U.S. Patent No. 5,919,452 is sufficient to qualify as prior art, then US Patent No. 5,919,452 is sufficient to enable the claims. As indicated above, treatment of all TNFα-mediated diseases is enabled by the specification. Thus, one of ordinary skill in the art would not be subject to undue experimentation in using the claimed invention. Reconsideration and withdrawal of the rejection are respectfully requested.

Objection to Claim 15 under 37 C.F.R. § 1.75

The Examiner has objected to Claim 15 under 37 C.F.R. § 1.75 as being a substantial duplicate of Claim 11. The Examiner states that "[w]hen two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claims ."

Applicants have amended Claim 11 to be directed to treatment of TNFα-mediated hepatitis. In contrast, Claim 15 is directed to treatment of inflammation associated with TNFα-mediated hepatitis. Reconsideration and withdrawal of the objection are respectfully requested.

Rejection to Claims 1, 3-5, 11-12 and 14-15 Under 35 U.S.C. § 112, second paragraph

The Examiner has rejected Claims 1, 3-5, 11-12 and 14-15 as indefinite in the use of "cA2" as the sole means of identifying the claimed antibody. Specifically, the Examiner states that "[t]he use of 'cA2' monoclonal antibody as the sole means of identifying the claimed antibody renders the claim indefinite because 'cA2' is merely a laboratory designation which does not clearly define the claimed product, since different laboratories may use the same laboratory designation [ ] to define completely distinct hybridomas / cell lines."

Applicants respectfully traverse this rejection. cA2 is not used as the sole means of identifying the antibody in the claims. The claims and specification provide a great deal of

description regarding cA2's structure and properties. As amended, the claims explicitly state that the antibody is a chimeric anti-TNFα monoclonal antibody. Further, the specification clearly discloses that the antibody is a chimeric anti-TNFα monoclonal antibody, and provides a detailed disclosure of the production, structure and function of cA2. (Specification at page 17, lines 2-8; page 19, lines 7-16; page 26, lines 21-28 and page 34, line 12 to page 35, line 4) For instance, Examples I-IX teach the production, characterization and expression of the cA2 antibody and Examples X-XII teach assays for screening the cA2 antibody for specificity and efficacy.

Moreover, "cA2" is recognized by those skilled in the art as a unique identifier of Applicants' chimeric anti-TNFα monoclonal antibody. A number of scientific articles and press releases refer to Applicants' claimed monoclonal antibody as "cA2." (See, for example, Elliott, M. J. *et al.*, "Treatment of Rheumatoid Arthritis with Chimeric Monoclonal Antibodies to Tumor Necrosis Factor α," *Arthritis Rheum, 36*:1681-1690 (1993) (Exhibit C); Walker, R.E., "Inhibition of Immunoreactive Tumor Necrosis Factor-alpha by a Chimeric Antibody in Patients Infected with Human Immunodeficiency Virus Type 1," J. *Infect. Dis., 174*(1):63-8 (1996), abstract from AIDSLINEMED/96261994 (Exhibit D); and "New Monoclonal Antibody Effective Treatment For Crohn's Disease Therapy," Doctor's Guide (May 13, 1997), http://www.docguide.com/dg.nsf/PrintPrint/815D53A771190A4285256496004B0796 (Exhibit E)). These references are representative of the general knowledge of one skilled in the art and demonstrate that the identifier "cA2" clearly defines the claimed product. Thus, the cA2 antibody is well known in the art.

Moreover, a number of claims have issued which refer to the instant chimeric anti-TNFα monoclonal antibody as cA2. For example, the claims of related U.S. Patent No. 6,284,471, which has the same priority date and has a substantially identical specification as the instant application, recite cA2. (A copy of the claim set of U.S. Patent No. 6,284,471 is attached hereto as "Exhibit F" for the Examiner's convenience).

Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection to Claims 1-15 Under 35 U.S.C. § 112, second paragraph

The Examiner has rejected Claims 1-15 as indefinite in the recitation of 'hepatitis pathologies' because the metes and bounds of said 'pathologies' are ill-defined and ambiguous."

The Examiner further states that "[t]here is insufficient description of the nature and targeted endpoints of treating 'hepatitis pathologies' to apprise the ordinary artisan of the metes and bound of the claimed 'hepatitis pathologies'."

Applicants respectfully disagree. Hepatitis is defined as inflammation of the liver from any cause. *See* The Merck Manual of Medical Information, Berkow *et al.*, Pocket Books, pages 571-574 (1997) (Exhibit A). This definition of hepatitis is representative of the general knowledge of one skilled in the art and demonstrates that the metes and bounds of hepatitis pathologies is not ill-defined and ambiguous. Nonetheless, to expedite prosecution, Applicants have amended Claims 1, 3-5, 7-8, 11-12 and 14-15 to recite "TNFα-mediated hepatitis." The term "pathologies" has been deleted. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection to Claims 1-15 Under 35 U.S.C. § 102(b)

The Examiner has rejected Claims 1-15 under 35 U.S.C. § 102(b) as being anticipated by Le *et al.* (U.S. Patent No. 5,919,452). The Examiner states that:

> Le et al. teach methods of treating TNF-α-mediated diseases, including alcohol-induced hepatitis (see column 35, line 12) with TNF-α-specific antibodies, including recombinant and chimeric antibodies and the cA2 antibody specificity of the instant invention... Applicant is reminded that no more of the reference is required than that it sets forth the substance of the invention. The claimed functional limitations would be inherent properties of the referenced methods to treat alcohol-induced hepatitis with recombinant cA2-specific antibodies. A species anticipates a claim to a genus. See MPEP 2131.02.

Applicants respectfully disagree. First, Applicants note that the Examiner has cited as prior art one of Applicants' priority patents (U.S. Patent No. 5,919,452). Le *et al.* (U.S. Patent No. 5,919,452) is not prior art under 35 U.S.C. § 102 (b) because it was not published more than one year before Applicants' priority date. Applicants are entitled to a priority date before U.S. Patent No. 5,919,452. Specifically, as discussed above, Applicants are entitled to priority to U.S. Application Serial No. 07/670,827 (filed March 18, 1991). Furthermore, the subject application is substantially identical to U.S. Patent No. 5,919,452, lists the same inventors and claims the benefit of priority to the same U.S. priority application (U.S. Serial No. 07/670,827) as U.S.

Serial No. 07/670,827) as U.S. Patent No. 5,919,452. Hence, Le *et al.* U.S. Patent No. 5,919,452 is not prior art.

To qualify as prior art, a reference must meet the requirement of enablement. As stated in the MPEP at § 2121.01:

> "In determining that quantum of prior art disclosure which is necessary to declare an applicant's invention 'not novel' or 'anticipated' within section 102, the stated test is whether a reference contains an 'enabling disclosure'...."

(Quoting *In re Hoeksema*, 399 F.2d 269, 158 USPQ 596 (CCPA 1968).

> The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation.

(Citing *Elan Pharm. Inc. v. Mayo Foundation for Medical and Education Research*, 346 F.3d 1051, 1054, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003).

Therefore, if Le *et al. 's* disclosure in Applicants' priority patent, U.S. Patent No. 5,919,452, is sufficient to qualify as prior art, then the U.S. Patent No. 5,919,452 priority patent is sufficient to support the claims, and the claims, at the very least, are entitled to the benefit of priority to the filing date of February 4, 1994. Hence, Le *et al.* U.S. Patent No. 5,919,452 is not prior art. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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Dated: April 7, 2005

res treatment with antibiotics. Narrowed can be dilated by an endoscopic or surgical :dure. Cancer of the bile ducts (cholangio- ıoma) develops in 10 to 15 percent of the le with primary sclerosing cholangitis. The r is slow-growing, and treatment entails an endoscopic procedure to place stents ; bile ducts to open up the diseased ducts. sionally, surgery is required.

lpha$_1$-Antitrypsin Deficiency

ı$_1$-antitrypsin deficiency is a disorder in which editary deficiency of alpha$_1$-antitrypsin may lung and liver disease.

ıha$_1$-antitrypsin, an enzyme produced by the is present in saliva, duodenal fluid, lung se- ms, tears, nasal secretions, and cerebrospi- ıid. This enzyme inhibits the action of other nes that break down proteins. A lack of ı-antitrypsin allows the other enzymes to ge tissue in the lungs. The deficiency in ! represents a failure of the liver to secrete nzyme. Its retention inside liver cells may ; damage, fibrosis (scarring), and cirrhosis.

ptoms and Prognosis

to 25 percent of children with alpha$_1$-anti- in deficiency develop cirrhosis and portal rtension and die before age 12. About 25 per- lie by age 20. Another 25 percent have only r liver abnormalities and live into adulthood. emaining 25 percent have no evidence of essive disease.

ıha$_1$-antitrypsin deficiency is uncommon in s and, even if present, may not cause cirrho- lore commonly, adults with the disorder de- emphysema, a lung disease that results in asing shortness of breath. Liver cancer may ually develop.

tment

olacement therapy using synthetic alpha$_1$- ypsin has shown some promise, but liver plantation remains the only successful treat- . Liver damage does not usually recur in the planted liver, which produces alpha$_1$-anti- in.

atment in adults is usually directed at the disease. Measures include preventing infec- and getting a person who smokes to stop ing.

Hepatitis

Hepatitis is inflammation of the liver from any cause.

Hepatitis commonly results from a virus, particularly one of five hepatitis viruses—A, B, C, D, or E. Less commonly, hepatitis results from other viral infections, such as infectious mononucleosis, yellow fever, and cytomegalovirus infection. The major nonviral causes of hepatitis are alcohol and drugs. Hepatitis can be acute (lasting less than 6 months) or chronic; it occurs commonly throughout the world.

Hepatitis A virus spreads primarily from the stool of one person to the mouth of another. Such transmission is usually the result of poor hygiene. Waterborne and foodborne epidemics are common, especially in developing countries. Eating contaminated raw shellfish is sometimes responsible. Isolated cases, usually arising from person-to-person contact, are also common. Most hepatitis A infections cause no symptoms and go unrecognized.

Hepatitis B virus is less easily transmitted than hepatitis A virus. One way it can be transmitted is through contaminated blood or blood products. However, because of precautions taken to ensure a safe blood supply, blood transfusions rarely are responsible for the transmission of the hepatitis B virus in the United States. Transmission commonly occurs among injecting drug users who share needles, as well as between sexual partners, both heterosexual and male homosexual. A pregnant woman infected with hepatitis B can transmit the virus to her baby during birth. The risk of exposure to the hepatitis B virus is increased for patients undergoing kidney dialysis or in cancer units and for hospital personnel who have contact with blood. Also at risk are people in closed environments (such as prisons and institutions for the mentally retarded), where close personal contact exists.

Hepatitis B can be transmitted by healthy people who are chronic carriers of the virus. Whether insect bites can transmit this virus isn't clear. Many cases of hepatitis B have no known source. In areas of the world such as the Far East and parts of Africa, hepatitis B virus is responsible for many cases of chronic hepatitis, cirrhosis, and liver cancer.

Hepatitis C virus causes at least 80 percent of the hepatitis cases arising from blood transfusions, plus many scattered cases of acute hepatitis. It is most commonly transmitted by injecting drug users who share needles. Sexual transmission is uncommon. Hepatitis C virus is responsible for many cases of chronic hepatitis and some cases of cirrhosis and liver cancer. For unknown reasons, people with alcoholic liver disease often have hepatitis C as well; the combination of diseases sometimes produces a greater loss of liver function than would result from either disease alone. A small proportion of healthy people appear to be chronic carriers of the hepatitis C virus.

Hepatitis D virus occurs only as a co-infection with hepatitis B virus, and it makes the hepatitis B infection more severe. Drug addicts are at relatively high risk.

Hepatitis E virus causes occasional epidemics similar to those caused by hepatitis A virus. So far, these epidemics have occurred only in underdeveloped countries.

Acute Viral Hepatitis

Acute viral hepatitis is inflammation of the liver caused by infection with one of the five hepatitis viruses; for most people the inflammation begins suddenly and lasts only a few weeks.

Symptoms and Diagnosis

Symptoms of acute viral hepatitis usually begin suddenly. They include a poor appetite, a feeling of being ill, nausea, vomiting, and often a fever. In people who smoke, a distaste for cigarettes is a typical symptom. Occasionally, especially with hepatitis B infection, the person develops joint pains and wheals (itchy red hives on the skin).

After a few days, the urine becomes dark, and jaundice may develop. Most symptoms typically disappear at this point and the person feels better even though the jaundice is getting worse. Symptoms of cholestasis (a stoppage or reduction of bile flow)▲—such as pale stools and general itch-

▲ see page 561

ing—may develop. The jaundice usually peaks in 1 to 2 weeks, then fades over 2 to 4 weeks.

Acute viral hepatitis is diagnosed on the basis of the person's symptoms and the results of blood tests that evaluate liver function. In about half the people with this disease, a doctor will find the liver to be tender and somewhat enlarged.

Acute viral hepatitis must be distinguished from several other conditions that cause similar symptoms. For instance, the flulike symptoms early in the disease can mimic those of other viral diseases, such as influenza and infectious mononucleosis. Fever and jaundice are also symptoms of alcoholic hepatitis, which occurs in people who regularly drink significant amounts of alcohol.▲ The specific diagnosis of acute viral hepatitis can be made if blood tests reveal viral proteins or antibodies against hepatitis viruses.

Prognosis

Acute viral hepatitis can produce anything from a minor flulike illness to fatal liver failure. In general, hepatitis B is more serious than hepatitis A and is occasionally fatal, especially in elderly people. The course of hepatitis C is somewhat unpredictable: The acute illness is usually mild, but liver function may improve and then worsen repeatedly for several months.

A person with acute viral hepatitis usually recovers after 4 to 8 weeks, even without treatment. Hepatitis A rarely if ever becomes chronic. Hepatitis B becomes chronic in 5 to 10 percent of the infected people and can be mild or full-blown. Hepatitis C has the greatest likelihood of becoming chronic—about a 75 percent chance. Though usually mild and often without symptoms, hepatitis C is a serious problem because about 20 percent of the affected people eventually develop cirrhosis.

A person with acute viral hepatitis can become a chronic carrier of the virus. In the carrier state, the person has no symptoms but is still infected. This situation occurs only with hepatitis B and C viruses, not hepatitis A virus. A chronic carrier may eventually develop liver cancer.

▲ see page 566

Treatment

People with unusually severe acute hepatitis may require hospitalization, but in most cases treatment isn't necessary. After the first several days, appetite usually returns and the person doesn't need to stay in bed. Severe restrictions of diet or activity are unnecessary, and vitamin supplements are not required. Most people can safely return to work after the jaundice clears, even if their liver function test results aren't quite normal.

Prevention

Good hygiene helps prevent the spread of hepatitis A virus. Because the stool of people with hepatitis A is infectious, stool samples must be handled with special care by health practitioners. The same is true for the blood of people with any type of acute hepatitis. On the other hand, infected people don't require isolation—it does little to prevent the transmission of hepatitis A, and it won't prevent the transmission of hepatitis B or C.

Medical personnel reduce the chance of infection from blood transfusions by avoiding unnecessary transfusions, using blood donated by volunteers rather than paid donors, and screening all blood donors for hepatitis B and C. Because of screening, the number of cases of hepatitis B and C transmitted through a blood transfusion has been greatly reduced, though not eliminated.

Vaccination against hepatitis B stimulates the body's immune defenses and protects most people well. However, dialysis patients, people with cirrhosis, and people with an impaired immune system derive less protection from vaccination. Vaccination is especially important for people at risk of contracting hepatitis B, though it isn't effective once the disease is established. For these various reasons, universal vaccination of all people against hepatitis B is being increasingly recommended.

Hepatitis A vaccines are given to people at high risk of acquiring the infection, such as travelers to parts of the world where the disease is widespread. No vaccines are available against hepatitis C, D, and E viruses.

People who haven't been vaccinated and who are exposed to hepatitis may receive an antibody preparation (immune serum globulin) for protection. Antibodies are intended to give immediate

protection against viral hepatitis, but the amou of protection varies greatly with different situ tions. For people who have been exposed—p haps by an accidental needlestick—to blood fected with hepatitis B virus, hepatitis B immu globulin provides better protection than ordina immune serum globulin. Infants born to mothe with hepatitis B are given hepatitis B immu globulin and are vaccinated. This combinati prevents chronic hepatitis B in about 70 perce of those infants.

Chronic Hepatitis

Chronic hepatitis is inflammation of the liver th lasts at least 6 months.

Chronic hepatitis, though much less comm than acute hepatitis, can persist for years, ev decades. It is usually quite mild and doesn't p duce any symptoms or significant liver dama In some cases, though, continued inflammati slowly damages the liver, eventually produci cirrhosis and liver failure.

Causes

Hepatitis C virus is a common cause of chroi hepatitis; about 75 percent of acute hepatitis cases become chronic. Hepatitis B virus, son times with hepatitis D virus, causes a smaller p centage of chronic infections. Hepatitis A and viruses do not cause chronic hepatitis. Dru such as methyldopa, isoniazid, nitrofuranto and possibly acetaminophen can also cau chronic hepatitis, particularly when they a taken for prolonged periods. Wilson's disease rare hereditary disease involving abnormal co per retention,▲ may cause chronic hepatitis children and young adults.

No one knows exactly why the same virus and drugs will cause chronic hepatitis in soi people but not in others, or why the degree severity varies. One possible explanation is th in people who develop chronic hepatitis, the i mune system overreacts to the viral infection drug.

In many people with chronic hepatitis, no o vious cause can be found. In some of these peop there appears to be an overactive immune syste reaction that is responsible for the chronic infla mation. This condition, called **autoimmune hep titis**, is more common among women than men

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People who haven't been vaccinated and who are exposed to hepatitis may receive an antibody preparation (immune serum globulin) for protection. Antibodies are intended to give immediate protection against viral hepatitis, but the amount of protection varies greatly with different situations. For people who have been exposed—perhaps by an accidental needlestick—to blood infected with hepatitis B virus, hepatitis B immune globulin provides better protection than ordinary immune serum globulin. Infants born to mothers with hepatitis B are given hepatitis B immune globulin and are vaccinated. This combination prevents chronic hepatitis B in about 70 percent of those infants.

Chronic Hepatitis

Chronic hepatitis is inflammation of the liver that lasts at least 6 months.

Chronic hepatitis, though much less common than acute hepatitis, can persist for years, even decades. It is usually quite mild and doesn't produce any symptoms or significant liver damage. In some cases, though, continued inflammation slowly damages the liver, eventually producing cirrhosis and liver failure.

Causes

Hepatitis C virus is a common cause of chronic hepatitis; about 75 percent of acute hepatitis C cases become chronic. Hepatitis B virus, sometimes with hepatitis D virus, causes a smaller percentage of chronic infections. Hepatitis A and E viruses do not cause chronic hepatitis. Drugs such as methyldopa, isoniazid, nitrofurantoin, and possibly acetaminophen can also cause chronic hepatitis, particularly when they are taken for prolonged periods. Wilson's disease, a rare hereditary disease involving abnormal copper retention,▲ may cause chronic hepatitis in children and young adults.

No one knows exactly why the same viruses and drugs will cause chronic hepatitis in some people but not in others, or why the degree of severity varies. One possible explanation is that in people who develop chronic hepatitis, the immune system overreacts to the viral infection or drug.

In many people with chronic hepatitis, no obvious cause can be found. In some of these people, there appears to be an overactive immune system reaction that is responsible for the chronic inflammation. This condition, called **autoimmune hepatitis**, is more common among women than men.

Symptoms and Diagnosis

About a third of chronic hepatitis cases develop after a bout of acute viral hepatitis. The remainder develop gradually without any obvious previous illness.

Many people with chronic hepatitis have no symptoms at all. For those who do, the symptoms often include a feeling of illness, poor appetite, and fatigue. Sometimes the person also has a low fever and some upper abdominal discomfort. Jaundice may or may not develop. Features of chronic liver disease may eventually develop. These can include an enlarged spleen, spiderlike blood vessels in the skin, and fluid retention. Other features may occur, especially in young women with autoimmune hepatitis. These can involve virtually any body system and include acne, cessation of menstrual periods, joint pain, lung scarring, inflammation of the thyroid gland and kidneys, and anemia.

Although the person's symptoms and liver function test results provide helpful diagnostic information, a liver biopsy (removal of a tissue sample for examination under a microscope) ■ is essential for a definite diagnosis. Examining liver tissue under a microscope allows a doctor to determine the severity of the inflammation and whether any scarring or cirrhosis has developed. The biopsy may also reveal the underlying cause of the hepatitis.

Prognosis and Treatment

Many people have chronic hepatitis for years without developing progressive liver damage. For others, the disease gradually worsens. When this happens and the disease is the result of viral hepatitis B or C infection, the antiviral agent interferon-alpha may stop the inflammation. However, the drug is expensive, adverse effects are common, and hepatitis tends to recur once treatment is stopped. Therefore, such treatment is reserved for selected people with the infection. Ribavirin with interferon-alpha may be a better treatment.

Autoimmune hepatitis is usually treated with corticosteroids, sometimes together with azathioprine. These drugs suppress the inflammation, resolve the symptoms, and improve long-

▲ see page 662

■ see page 560

term survival. Nevertheless, scarring (fibrosis) in the liver may gradually worsen. Discontinuing therapy usually leads to a recurrence, so most people have to take the drugs indefinitely. Over a period of years, about 50 percent of the people with autoimmune hepatitis develop cirrhosis, liver failure, or both.

If a drug is suspected to cause the hepatitis, the person should stop taking it. Doing so may make the chronic hepatitis disappear.

Regardless of the cause or type of chronic hepatitis, any complications—such as ascites (fluid in the abdominal cavity)▲ or encephalopathy (abnormal brain function)■—require treatment.

CHAPTER 119

Blood Vessel Disorders of the Liver

The liver receives a quarter of its blood supply from the hepatic artery, which comes from the heart. The other three quarters of its blood supply comes from the portal vein, which drains the intestine. Blood draining from the intestine is filled with digested food substances for the liver to process.

Blood leaves the liver through the hepatic vein. This blood is a mixture of blood from the hepatic artery and blood from the portal vein. The hepatic vein drains into the vena cava—the largest vein in the body—which then empties into the heart.

Abnormalities of the Hepatic Artery

The hepatic artery provides the only blood supply to certain parts of the liver, particularly the supporting tissue and the walls of the bile ducts. Narrowing or blockage of the artery or its branches can cause considerable damage to these areas. Flow through the artery may be disrupted by an injury, such as a gunshot wound or surgical trauma, or by a blood clot. Blood clots generally are caused by inflammation of the arterial wall (arteritis), or by an infusion of anticancer drugs or other toxic or irritating substances into the artery.

Aneurysms can also affect the hepatic artery. Aneurysms are a bulge at a weak spot in an artery; an aneurysm in the hepatic artery is usually caused by infection, arteriosclerosis, injury, or polyarteritis nodosa. An aneurysm that presses on a nearby bile duct may narrow or even block it, and jaundice may develop because bile flow from the liver backs up. As many as three quarters of these aneurysms rupture, often causing massive bleeding. An aneurysm may be treated by inserting a catheter into the hepatic artery and injecting an irritating substance that causes a blockage. If this procedure (called embolization) fails, surgery is performed to repair the artery.

Veno-occlusive Disease

Veno-occlusive disease is blockage of the small veins in the liver.

Veno-occlusive disease may occur at any age, but children ages 1 to 3 are particularly vulnerable because they have smaller blood vessels. Blockage may be caused by drugs and other substances toxic to the liver, such as *Senecio* leaves (used in Jamaica to make herbal tea), dimethylnitrosamine, aflatoxin, and anticancer drugs such as azathioprine. Radiation therapy also can produce a blockage of the small veins, as can antibodies produced during rejection of a liver transplant.★

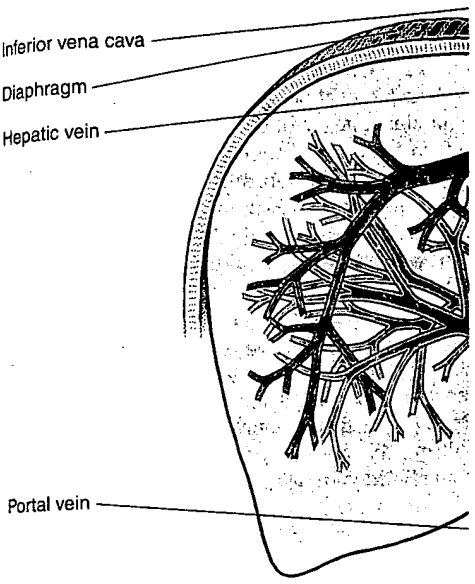
A blockage causes a backup of blood in the liver, reducing the liver's blood supply. The insufficient blood supply, in turn, damages the liver cells.

▲ see page 564

■ see page 564

★ see page 835

Blood Supply of the Liver



Symptoms, Prognosis, and Treatmer

Blockage of the small veins causes the liver swell with blood, making it tender to the tou Fluid may leak from the surface of the swol liver and accumulate in the abdomen, produc a condition called ascites.▲ The backup of blc in the liver also raises the pressure in the por vein (a condition called portal hypertension and in the veins that empty into it. This higl pressure may cause varicose veins in the eso agus (esophageal varices), which may rupt and hemorrhage.

Typically, a blockage disappears quickly, a the person recovers with or without treatm However, some people die of liver failure.★ others, the pressure in the portal vein rema high and the injury leads to cirrhosis.● The c treatment is to stop taking the substance or d causing the blockage. The exact course of the ease depends on the extent of damage

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POCKET BOOKS, a division of Simon & Schuster, Inc.
1230 Avenue of the Americas, New York, NY 10020

Published by arrangement with Merck & Company, Inc.

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First Pocket Books trade paperback printing September 2000

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is given in relation to each disease
and a chapter on over-the-counter
been added to the Drugs section.

of Law will be entered on the same date herewith.

ORDER AND JUDGMENT

In accordance with the Findings of Fact and Conclusions of Law entered on the same date herewith,

IT IS HEREBY ORDERED AND ADJUDGED, as follows:

1. The Nolan patent (No. 4,506,189), issued on March 19, 1985, is a valid patent.

2. By the manufacture, production, sale and distribution of its SAF-T-COTE fluorescent lamp, Trojan has infringed the Nolan patent.

3. By virtue of this infringement, Shat-R-Shield is entitled to injunctive relief. Trojan shall immediately cease and desist from the manufacture, production, sale and distribution of the SAF-T-COTE fluorescent lamp.

4. Trojan shall recall all the SAF-T-COTE fluorescent lamps sold to and still in the possession of its customers.

5. The Court having determined that Trojan's infringement was not willful and wanton, Shat-R-Shield is not entitled to treble damages.

6. Shat-R-Shield shall have no accounting for monetary damages.

7. The Court having found that this is not an exceptional case, Shat-R-Shield is not entitled to its attorney's fees.

8. All claims having been resolved as to all parties herein, this action is now DISMISSED and STRICKEN from the docket.

9. There being no just reason for delay, this is a FINAL and APPEALABLE Order and Judgment.

Court of Appeals, Federal Circuit

In re Wands

No. 87-1454

Decided September 30, 1988

PATENTS

1. Patentability/Validity — Adequacy of disclosure (§115.12)

Data disclosed in application for immunoassay method patent, which shows that applicants screened nine of 143 cell lines developed for production of antibody necessary to practice invention, stored remainder of said cell lines, and found that four out of nine cell lines screened produced antibody falling within limitation of claims, were erroneously interpreted by Board of Patent Appeals and Interferences as failing to meet disclosure requirements of 35 USC 112, since board's characterization of stored cell lines as "failures" demonstrating unreliability of applicants' methods was improper in view of fact that such unscreened cell lines prove nothing concerning probability of success of person skilled in art attempting to obtain requisite antibodies using applicants' methods.

2. Patentability/Validity — Adequacy of disclosure (§115.12)

Disclosure in application for immunoassay method patent does not fail to meet enablement requirement of 35 USC 112 by requiring "undue experimentation," even though production of monoclonal antibodies necessary to practice invention first requires production and screening of numerous antibody producing cells or "hybridomas," since practitioners of art are prepared to screen negative hybridomas in order to find those that produce desired antibodies, since in monoclonal antibody art one "experiment" is not simply screening of one hybridoma but rather is entire attempt to make desired antibody, and since record indicates that amount of effort needed to obtain desired antibodies is not excessive, in view of applicants' success in each attempt to produce antibody that satisfied all claim limitations.

Appeal from decision of Patent and Trademark Office, Board of Patent Appeals and Interferences.

Application for patent of Jack R. Wands, Vincent R. Zurawski, Jr., and Hubert J. P. Schoemaker, serial number 188,735. From decision of Board of Patent Appeals and Interferences affirming rejection of application, applicants appeal. Reversed; Newman, J., concurring in part and dissenting in part in separate opinion.

Jorge A. Goldstein, of Saidman, Sterne, Kessler & Goldstein (Henry N. Wixon, with them on brief), Washington, D.C., for appellant.

John H. Raubitschek, associate solicitor (Joseph F. Nakamura and Fred E. McKelvey, with him on brief), PTO, for appellee.

Before Smith, Newman, and Bissell, circuit judges.

Smith, J.

This appeal is from the decision of the Patent and Trademark Office (PTO) Board of Patent Appeals and Interferences (board) affirming the rejection of all remaining claims in appellant's application for a patent, serial No. 188,735, entitled "Immunoassay Utilizing Monoclonal High Affinity IgM

Antibodies," which was filed September 19, 1980.[1] The rejection under 35 U.S.C. §112, first paragraph, is based on the grounds that appellant's written specification would not enable a person skilled in the art to make the monoclonal antibodies that are needed to practice the claimed invention without undue experimentation. We reverse.

I. *Issue*

The only issue on appeal is whether the board erred, as a matter of law, by sustaining the examiner's rejection for lack of enablement under 35 U.S.C. §112, first paragraph, of all remaining claims in appellants' patent application, serial No. 188,735.

II. *Background*

A. *The Art.*

The claimed invention involves immunoassay methods for the detection of hepatitis B surface antigen by using high-affinity monoclonal antibodies of the IgM isotype. *Antibodies* are a class of proteins (immunoglobulins) that help defend the body against invaders such as viruses and bacteria. An antibody has the potential to bind tightly to another molecule, which molecule is called an antigen. The body has the ability to make millions of different antibodies that bind to different antigens. However, it is only after exposure of an antigen that a complicated *immune response* leads to the production of antibodies against that antigen. For example, on the surface of hepatitis B virus particles there is a large protein called *hepatitis B surface antigen* (HBsAg). As its name implies, it is capable of serving as an antigen. During a hepatitis B infection (or when purified HBsAg is injected experimentally), the body begins to make antibodies that bind tightly and specifically to HBsAg. Such antibodies can be used as reagents for sensitive diagnostic tests (*e.g.*, to detect hepatitis B virus in blood and other tissues, a purpose of the claimed invention). A method for detecting or measuring antigens by using antibodies as reagents is called an *immunoassay*.

Normally, many different antibodies are produced against each antigen. One reason for this diversity is that different antibodies are produced that bind to different regions (determinants) of a large antigen molecule such as HBsAg. In addition, different antibodies may be produced that bind to the same determinant. These usually differ in the tightness with which they bind to the determinant. *Affinity* is a quantitative measure of the strength of antibody-antigen binding. Usually an antibody with a higher affinity for an antigen will be more useful for immunological diagnostic tests than one with a lower affinity. Another source of heterogeneity is that there are several immunoglobulin classes or *isotypes*. Immunoglobulin G (IgG) is the most common isotype in serum. Another isotype, immunoglobulin M (IgM), is prominent early in the immune response. IgM molecules are larger than IgG molecules, and have 10 antigen-binding sites instead of the 2 that are present in IgG. Most immunoassay methods use IgG, but the claimed invention uses only IgM antibodies.

For commercial applications there are many disadvantages to using antibodies from serum. Serum contains a complex mixture of antibodies against the antigen of interest within a much larger pool of antibodies directed at other antigens. There are available only in a limited supply that ends when the donor dies. The goal of monoclonal antibody technology is to produce an unlimited supply of a single purified antibody.

The blood cells that make antibodies are *lymphocytes*. Each lymphocyte makes only one kind of antibody. During an immune response, lymphocytes exposed to their particular antigen divide and mature. Each produces a *clone* of identical daughter cells, all of which secrete the same antibody. Clones of lymphocytes, all derived from a single lymphocyte, could provide a source of a single homogeneous antibody. However, lymphocytes do not survive for long outside of the body in cell culture.

Hybridoma technology provides a way to obtain large numbers of cells that all produce the same antibody. This method takes advantage of the properties of *myeloma* cells derived from a tumor of the immune system. The cancerous myeloma cells can divide indefinitely in vitro. They also have the potential ability to secrete antibodies. By appropriate experimental manipulations, a myeloma cell can be made to fuse with a lymphocyte to produce a single hybrid cell (hence, a hybridoma) that contains the genetic material of both cells. The hybridoma secretes the same antibody that was made by its parent lymphocyte, but acquires the capability of the myeloma cell to divide and grow indefinitely in cell culture. Antibodies produced by a clone of hybridoma cells (i.e., by hybridoma

[1] *In re Wands*, Appeal No. 673-76 (Bd. Pat. App. & Int. Dec. 30, 1986).

cells that are all progeny of a single cell) are called monoclonal antibodies.[2]

B. *The Claimed Invention.*

The claimed invention involves methods for the immunoassay of HBsAg by using high-affinity monoclonal IgM antibodies. Jack R. Wands and Vincent R. Zurawski, Jr., two of the three coinventors of the present application, disclosed methods for producing monoclonal antibodies against HBsAg in United States patent No. 4,271,145 (the '145 patent), entitled "Process for Producing Antibodies to Hepatitis Virus and Cell Lines Therefor," which patent issued on June 2, 1981. The '145 patent is incorporated by reference into the application on appeal. The specification of the '145 patent teaches a procedure for immunizing mice against HBsAg, and the use of lymphocytes from these mice to produce hybridomas that secrete monoclonal antibodies specific for HBsAg. The '145 patent discloses that this procedure yields both IgG and IgM antibodies with high-affinity binding to HBsAg. For the stated purpose of complying with the best mode requirement of 35 U.S.C. §112, first paragraph, a hybridoma cell line that secretes IgM antibodies against HBsAg (the 1F8 cell line) was deposited at the American Type Culture Collection, a recognized cell depository, and became available to the public when the '145 patent issued.

The application on appeal claims methods for immunoassay of HBsAg using monoclonal antibodies such as those described in the '145 patent. Most immunoassay methods have used monoclonal antibodies of the IgG isotype. IgM antibodies were disfavored in the prior art because of their sensitivity to reducing agents and their tendency to self-aggregate and precipitate. Appellants found that their monoclonal IgM antibodies could be used for immunoassay of HbsAg with unexpectedly high sensitivity and specificity. Claims 1, 3, 7, 8, 14, and 15 are drawn to methods for the immunoassay of HBsAg using high-affinity IgM monoclonal antibodies. Claims 19 and 25–27 are for chemically modified (*e.g.*, radioactively labeled) monoclonal IgM antibodies used in the assays. The broadest method claim reads:

> 1. An immunoassay method utilizing an antibody to assay for a substance comprising hepatitis B-surface antigen (HBsAg) determinants which comprises the steps of:
>
> contacting a test sample containing said substance comprising HBsAg determinants with said antibody; and
>
> determining the presence of said substance in said sample;
>
> wherein said antibody is a monoclonal high affinity IgM antibody having a binding affinity constant for said HBsAg determinants of at least 10^9 M^{-1}.

Certain claims were rejected under 35 U.S.C. §103; these rejections have not been appealed. Remaining claims 1, 3, 7, 8, 14, 15, 19, and 25–27 were rejected under 35 U.S.C. §112, first paragraph, on the grounds that the disclosure would not enable a person skilled in the art to make and use the invention without undue experimentation. The rejection is directed solely to whether the specification enables one skilled in the art to make the monoclonal antibodies that are needed to practice the invention. The position of the PTO is that data presented by Wands show that the production of high-affinity IgM anti-HBsAg antibodies is unpredictable and unreliable, so that it would require undue experimentation for one skilled in the art to make the antibodies.

III. *Analysis*

A. *Enablement by Deposit of Micro-organisms and Cell Lines.*

The first paragraph of 35 U.S.C. §112 requires that the specification of a patent must enable a person skilled in the art to make and use the claimed invention. "Patents * * * are written to enable those skilled in the art to practice the invention."[3] A patent need not disclose what is well known in the art.[4] Although we review underlying facts found by the board under a "clearly erroneous" standard,[5] we review enablement as a question of law.[6]

Where an invention depends on the use of living materials such as microorganisms or

[2] For a concise description of monoclonal antibodies and their use in immunoassay see *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1368–71, 231 USPQ 81, 82–83 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987).

[3] *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1556, 220 USPQ 303, 315 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

[4] *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

[5] *Coleman v. Dines*, 754 F.2d 353, 356, 224 USPQ 857, 859 (Fed. Cir. 1985).

[6] *Moleculon Research Corp. v. CBS, Inc.*, 793 F.2d 1261, 1268, 229 USPQ 805, 810 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 875 (1987); *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 960 n.6, 220 USPQ 592, 599 n.6 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 835 [225 USPQ 232] (1984).

cultured cells, it may be impossible to enable the public to make the invention (i.e., to obtain these living materials) solely by means of a written disclosure. One means that has been developed for complying with the enablement requirement is to deposit the living materials in cell depositories which will distribute samples to the public who wish to practice the invention after the patent issues.[7] Administrative guidelines and judicial decisions have clarified the conditions under which a deposit of organisms can satisfy the requirements of section 112.[8] A deposit has been held necessary for enablement where the starting materials (i.e., the living cells used to practice the invention, or cells from which the required cells can be produced) are not readily available to the public.[9] Even when starting materials are available, a deposit has been necessary where it would require undue experimentation to make the cells of the invention from the starting materials.[10]

In addition to satisfying the enablement requirement, deposit of organisms also can be used to establish the filing date of the application as the prima facie date of invention,[11] and to satisfy the requirement under 35 U.S.C. §114 that the PTO be guaranteed access to the invention during pendency of the application.[12] Although a deposit may serve these purposes, we recognized, in *In re Lundak*,[13] that these purposes, nevertheless, may be met in ways other than by making a deposit.

A deposit also may satisfy the best mode requirement of section 112, first paragraph, and it is for this reason that the 1F8 hybridoma was deposited in connection with the '145 patent and the current application. Wands does not challenge the statements by the examiner to the effect that, although the deposited 1F8 line enables the public to perform immunoassays with antibodies produced by that single hybridoma, the deposit does not enable the generic claims that are on appeal. The examiner rejected the claims on the grounds that the written disclosure was not enabling and that the deposit was inadequate. Since we hold that the written disclosure fully enables the claimed invention, we need not reach the question of the adequacy of deposits.

B. *Undue Experimentation.*

Although inventions involving microorganisms or other living cells often can be enabled by a deposit,[14] a deposit is not always necessary to satisfy the enablement requirement.[15] No deposit is necessary if the biological organisms can be obtained from readily available sources or derived from readily available starting materials through routine screening that does not require undue experimentation.[16] Whether the specification in an application involving living cells (here, hybridomas) is enabled without a deposit must be decided on the facts of the particular case.[17]

Appellants contend that their written specification fully enables the practice of

[7] *In re Argoudelis*, 434 F.2d 1390, 1392–93, 168 USPQ 99, 101–02 (CCPA 1970).

[8] *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985); *Feldman v. Aunstrup*, 517 F.2d 1351, 186 USPQ 108 (CCPA 1975), *cert. denied*, 424 U.S. 912 [188 USPQ 720] (1976); Manual of Patent Examining Procedure (MPEP) 608.01 (p)(C) (5th ed. 1983, rev. 1987). *See generally* Hampar, *Patenting of Recombinant DNA Technology: The Deposit Requirement*, 67 J. Pat. Trademark Off. Soc'y 569 (1985).

[9] *In re Jackson*, 217 USPQ 804, 807–08 (Bd. App. 1982) (strains of a newly discovered species of bacteria isolated from nature); *Feldman*, 517 F.2d 1351, 186 USPQ 108 (uncommon fungus isolated from nature); *In re Argoudelis*, 434 F.2d at 1392, 168 USPQ at 102 (novel strain of antibiotic-producing microorganism isolated from nature); *In re Kropp*, 143 USPQ 148, 152 (Bd. App. 1959) (newly discovered microorganism isolated from soil).

[10] *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (genetically engineered bacteria where the specification provided insufficient information about the amount of time and effort required); *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (unique cell line produced from another cell line by mutagenesis).

[11] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96; *In re Feldman*, 517 F.2d at 1355, 186 USPQ at 113; *In re Argoudelis*, 434 F.2d at 1394–96, 168 USPQ at 103–04 (Baldwin, J. concurring).

[12] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96; *In re Feldman*, 517 F.2d at 1354, 186 USPQ at 112.

[13] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96.

[14] *In re Argoudelis*, 434 F.2d at 1393, 168 USPQ at 102.

[15] *Tabuchi v. Nubel*, 559 F.2d 1183, 194 USPQ 521 (CCPA 1977).

[16] *Id.* at 1186–87, 194 USPQ at 525; *Merck & Co. v. Chase Chem. Co.*, 273 F.Supp. 68, 77, 155 USPQ 139, 146 (D.N.J. 1967); *Guaranty Trust Co. v. Union Solvents Corp.*, 54 F.2d 400, 403–06, 12 USPQ 47, 50–53 (D. Del. 1931), *aff'd*, 61 F.2d 1041, 15 USPQ 237 (3d Cir. 1932), *cert. denied*, 288 U.S. 614 (1933); MPEP 608.01(p)(C) ("No problem exists when the microorganisms used are known and readily available to the public.").

[17] *In re Jackson*, 217 USPQ at 807; *see In re Metcalfe*, 410 F.2d 1378, 1382, 161 USPQ 789, 792 (CCPA 1969).

their claimed invention because the monoclonal antibodies needed to perform the immunoassays can be made from readily available starting materials using methods that are well known in the monoclonal antibody art. Wands states that application of these methods to make high-affinity IgM anti-HBsAg antibodies requires only routine screening, and that does not amount to undue experimentation. There is no challenge to their contention that the starting materials (i.e., mice, HBsAg antigen, and myeloma cells) are available to the public. The PTO concedes that the methods used to prepare hybridomas and to screen them for high-affinity IgM antibodies against HBsAg were either well known in the monoclonal antibody art or adequately disclosed in the '145 patent and in the current application. This is consistent with this court's recognition with respect to another patent application that methods for obtaining and screening monoclonal antibodies were well known in 1980.[18] The sole issue is whether, in this particular case, it would require undue experimentation to produce high-affinity IgM monoclonal antibodies.

Enablement is not precluded by the necessity for some experimentation such as routine screening.[19] However, experimentation needed to practice the invention must not be undue experimentation.[20] "the key word is 'undue,' not 'experimentation.' "[21]

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. Ansul Co. v. Uniroyal, Inc. [448 F.2d 872, 878-79; 169 USPQ 759, 762-63 (2d Cir. 1971), *cert. denied*, 404 U.S. 1018 [172 USPQ 257] (1972)]. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed * * *.[22]

The term "undue experimentation" does not appear in the statute, but it is well established that enablement requires that the specification teach those in the art to make and use the invention without undue experimentation.[23] Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations. The board concluded that undue experimentation would be needed to practice the invention on the basis of experimental data presented by Wands. These data are not in dispute. However, Wands and the board disagree strongly on the conclusion that should be drawn from that data.

Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*.[24] They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.[25]

In order to understand whether the rejection was proper, it is necessary to discuss further the methods for making specific monoclonal antibodies. The first step for making monoclonal antibodies is to immunize an animal. The '145 patent provides a detailed description of procedures for immunizing a specific strain of mice against HBsAg. Next the spleen, an organ rich in lymphocytes, is removed and the lymphocytes are separated from the other spleen cells. The lymphocytes are mixed with myeloma cells, and the mixture is treated to cause a few of the cells to fuse with each other. Hybridoma cells that secrete the desired antibodies then must be isolated from the enormous number of other cells in the mixture. This is done through a series of screening procedures.

The first step is to separate the hybridoma cells from unfused lymphocytes and myeloma cells. The cells are cultured in a medi-

[18] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94.

[19] *Id.; Atlas Powder Co. v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984); *In re Angstadt*, 537 F.2d at 502-504, 190 USPQ at 218; *In re Geerdes*, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); *Mineral Separation, Ltd. v. Hyde*, 242 U.S. 261, 270-71 (1916).

[20] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *W.L. Gore*, 721 F.2d at 1557, 220 USPQ at 316; *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977) (Miller, J., concurring).

[21] *In re Angstadt*, 537 F.2d at 504, 190 USPQ at 219.

[22] *In re Jackson*, 217 USPQ at 807.

[23] *See Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *Atlas Powder*, 750 F.2d at 1576, 224 USPQ at 413.

[24] *Ex parte Forman*, 230 USPQ at 547.

[25] *Id.; see In re Colianni*, 561 F.2d at 224, 195 USPQ at 153 (Miller, J., concurring); *In re Rainer*, 347 F.2d 574, 577, 146 USPQ 218, 221 (CCPA 1965).

um in which all the lymphocytes and myeloma cells die, and only the hybridoma cells survive. The next step is to isolate and clone hybridomas that make antibodies that bind to the antigen of interest. Single hybridoma cells are placed in separate chambers and are allowed to grow and divide. After there are enough cells in the clone to produce sufficient quantities of antibody to analyze, the antibody is assayed to determine whether it binds to the antigen. Generally, antibodies from many clones do not bind the antigen, and these clones are discarded. However, by screening enough clones (often hundreds at a time), hybridomas may be found that secrete antibodies against the antigen of interest.

Wands used a commercially available radioimmunoassay kit to screen clones for cells that produce antibodies directed against HBsAg. In this assay the amount of radioactivity bound gives some indication of the strength of the antibody-antigen binding, but does not yield a numerical affinity constant, which must be measured using the more laborious Scatchard analysis. In order to determine which anti-HBsAg antibodies satisfy all of the limitations of appellants' claims, the antibodies require further screening to select those which have an IgM isotype and have a binding affinity constant of at least 10^9 M^{-1}.[26] The PTO does not question that the screening techniques used by Wands were well known in the monoclonal antibody art.

During prosecution Wands submitted a declaration under 37 C.F.R §1.132 providing information about all of the hybridomas that appellants had produced before filing the patent application. The first four fusions were unsuccessful and produced no hybridomas. The next six fusion experiments all produced hybridomas that made antibodies specific for HBsAg. Antibodies that bound at least 10,000 cpm in the commercial radioimmunoassay were classified as "high binders." Using this criterion, 143 high-binding hybridomas were obtained. In the declaration, Wands stated that [27]

> It is generally accepted in the art that, among those antibodies which are binders with 50,000 cpm or higher, there is a very high likelihood that high affinity (Ka [greater than] 10^9 M^{-1}) antibodies will be found. However, high affinity antibodies can also be found among high binders of between 10,000 and 50,000, as is clearly demonstrated in the Table.

The PTO has not challenged this statement.

The declaration stated that a few of the high-binding monoclonal antibodies from two fusions were chosen for further screening. The remainder of the antibodies and the hybridomas that produced them were saved by freezing. Only nine antibodies were subjected to further analysis. Four (three from one fusion and one from another fusion) fell within the claims, that is, were IgM antibodies and had a binding affinity constant of at least 10^9 M^{-1}. Of the remaining five antibodies, three were found to be IgG, while the other two were IgM for which the affinity constants were not measured (although both showed binding well above 50,000 cpm).

Apparently none of the frozen cell lines received any further analysis. The declaration explains that after useful high-affinity IgM monoclonal antibodies to HBsAg had been found, it was considered unnecessary to return to the stored antibodies to screen for more IgMs. Wands says that the existence of the stored hybridomas was disclosed to the PTO to comply with the requirement under 37 C.F.R. §1.56 that applicants fully disclose all of their relevant data, and not just favorable results.[28] How these stored hybridomas are viewed is central to the positions of the parties.

The position of the board emphasizes the fact that since the stored cell lines were not completely tested, there is no proof that any of them are IgM antibodies with a binding affinity constant of at least 10^9 M^{-1}. Thus, only 4 out of 143 hybridomas, or 2.8 percent, were *proved* to fall within the claims. Furthermore, antibodies that were proved to be high-affinity IgM came from only 2 of 10 fusion experiments. These statistics are viewed by the board as evidence that appellants' methods were not predictable or reproducible. The board concludes that Wands' low rate of demonstrated success shows that a person skilled in the art would have to

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[26] The examiner, the board, and Wands all point out that, technically, the strength of antibody-HBsAg binding is measured as *avidity*, which takes into account multiple determinants on the HBsAg molecule, rather than affinity. Nevertheless, despite this correction, all parties then continued to use the term "affinity." We will use the terminology of the parties. Following the usage of the parties, we will also use the term "high-affinity" as essentially synonymous with "having a binding affinity constant of at least 10^9 M^{-1}."

[27] A table in the declaration presented the binding data for antibodies from every cell line. Values ranged from 13,867 to 125,204 cpm, and a substantial proportion of the antibodies showed binding greater than 50,000 cpm. In confirmation of Dr. Wand's statement, two antibodies with binding less than 25,000 cpm were found to have affinity constants greater than 10^9 M^{-1}.

[28] *See Rohm & Haas Co. v. Crystal Chem. Co.*, 722 F.2d 1556, 220 USQ 98 (Fed. Cir. 1983).

engage in undue experimentation in order to make antibodies that fall within the claims.

Wands views the data quite differently. Only nine hybridomas were actually analyzed beyond the initial screening for HBsAg binding. Of these, four produced antibodies that fell within the claims, a respectable 44 percent rate of success. (Furthermore, since the two additional IgM antibodies for which the affinity constants were never measured showed binding in excess of 50,000 cpm, it is likely that these also fall within the claims.) Wands argues that the remaining 134 unanalyzed, stored cell lines should not be written off as failures. Instead, if anything, they represent partial success. Each of the stored hybridomas had been shown to produce a high-binding antibody specific for HBsAg. Many of these antibodies showed binding above 50,000 cpm and are thus highly likely to have a binding affinity constant of at least 10^9 M^{-1}. Extrapolating from the nine hybridomas that were screened for isotype (and from what is well known in the monoclonal antibody art about isotype frequency), it is reasonable to assume that the stored cells include some that produce IgM. Thus, if the 134 incompletely analyzed cell lines are considered at all, they provide some support (albeit without rigorous proof) to the view that hybridomas falling within the claims are not so rare that undue experimentation would be needed to make them.

The first four fusion attempts were failures, while high-binding antibodies were produced in the next six fusions. Appellants contend that the initial failures occurred because they had not yet learned to fuse cells successfully. Once they became skilled in the art, they invariably obtained numerous hybridomas that made high-binding antibodies against HBsAg and, in each fusion where they determined isotype and binding affinity they obtained hybridomas that fell within the claims.

Wands also submitted a second declaration under 37 C.F.R. §1.132 stating that after the patent application was submitted they performed an eleventh fusion experiment and obtained another hybridoma that made a high-affinity IgM anti-HBsAg antibody. No information was provided about the number of clones screened in that experiment. The board determined that, because there was no indication as to the number of hybridomas screened, this declaration had very little value. While we agree that it would have been preferable if Wands had included this information, the declaration does show that when appellants repeated their procedures they again obtained a hybridoma that produced an antibody that fit all of the limitations of their claims.

[1] We conclude that the board's interpretation of the data is erroneous. It is strained and unduly harsh to classify the stored cell lines (each of which was proved to make high-binding antibodies against HBsAg) as failures demonstrating that Wands' methods are unpredictable or unreliable.[29] At worst, they prove nothing at all about the probability of success, and merely show that appellants were prudent in not discarding cells that might someday prove useful. At best, they show that high-binding antibodies, the starting materials for IgM screening and Scatchard analysis, can be produced in large numbers. The PTO's position leads to the absurd conclusion that the more hybridomas an applicant makes and saves without testing the less predictable the applicant's results become. Furthermore, Wands' explanation that the first four attempts at cell fusion failed only because they had not yet learned to perform fusions properly is reasonable in view of the fact that the next six fusions were all successful. The record indicates that cell fusion is a technique that is well known to those of ordinary skill in the monoclonal antibody art, and there has been no claim that the fusion step should be more difficult or unreliable where the antigen is HBsAg than it would be for other antigens.

[2] When Wands' data is interpreted in a reasonable manner, analysis considering the factors enumerated in *Ex parte Forman* leads to the conclusion that undue experimentation would not be required to practice the invention. Wands' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known.

The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody. No evidence was presented by either party on how many hybridomas would be viewed by those in the art as requiring undue experimentation to screen. However, it seems unlikely that un-

[29] Even if we were to accept the PTO's 2.8% success rate, we would not be required to reach a conclusion of undue experimentation. Such a determination must be made in view of the circumstances of each case and cannot be made solely by reference to a particular numerical cutoff.

due experimentation would be defined in terms of the number of hybridomas that were never screened. Furthermore, in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen. This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics. Wands carried out this entire procedure three times, and was successful each time in making at least one antibody that satisfied all of the claim limitations. Reasonably interpreted, Wands' record indicates that, in the production of high-affinity IgM antibodies against HBsAG, the amount of effort needed to obtain such antibodies is not excessive. Wands' evidence thus effectively rebuts the examiner's challenge to the enablement of their disclosure.[30]

IV. *Conclusion*

Considering all of the factors, we conclude that it would not require undue experimentation to obtain antibodies needed to practice the claimed invention. Accordingly, the rejection of Wands' claims for lack of enablement under 35 U.S.C. §112, first paragraph, is reversed.

REVERSED

Newman, J., concurring in part, dissenting in part.

A

I concur in the court's holding that additional samples of hybridoma cell lines that produce these high-affinity IgM monoclonal antibodies need not be deposited. This invention, as described by Wands, is not a selection of a few rare cells from many possible cells. To the contrary, Wands states that all monoclonally produced IgM antibodies to hepatitis B surface antigen have the desired high avidity and other favorable properties, and that all are readily preparable by now-standard techniques.

Wands states that his United States Patent No. 4,271,145 describes fully operable techniques, and is distinguished from his first four failed experiments that are referred to in the Rule 132 affidavit. Wands argues that these biotechnological mechanisms are relatively well understood and that the preparations can be routinely duplicated by those of skill in this art, as in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987). I agree that it is not necessary that there be a deposit of multiple exemplars of a cell system that is readily reproduced by known, specifically identified techniques.

B

I would affirm the board's holding that Wands has not complied with 35 U.S.C. §112, first paragraph, in that he has not provided data sufficient to support the breadth of his generic claims. Wands' claims on appeal include the following:

> 19. Monoclonal high affinity IgM antibodies immunoreactive with HBsAg determinants, wherein said antibodies are coupled to an insoluble solid phase, and wherein the binding affinity constant of said antibodies for said HBsAg determinants is at least 10^9 M^{-1}.
>
> 26. Monoclonal high affinity IgM antibodies immunoreactive with hepatitis B surface antigen.

Wands states that he obtained 143 "high binding monoclonal antibodies of the right specificity" in the successful fusions; although he does not state how they were determined to be high binding or of the right specificity, for Wands also states that only nine of these 143 were tested.

Of these nine, four (three from one fusion and one from another fusion) were found to have the claimed high affinity and to be of the IgM isotype. Wands states that the other five were either of a different isotype or their affinities were not determined. (This latter statement also appears to contradict his statement that all 143 were "high binding".)

Wands argues that a "success rate of four out of nine", or 44.4%, is sufficient to support claims to the entire class. The Commissioner deems the success rate to be four out of 143, or 2.8%; to which Wands responds with statistical analysis as to how unlikely it is that Wands selected the only four out of 143 that worked. Wands did not, however, prove the right point. The question is whether Wands, by testing nine out of 143 (the Commissioner points out that the randomness of the sample was not established), and finding that four out of the nine had the desired properties, has provided sufficient experimental support for the breadth of the requested claims, in the context that "experi-

[30] *In re Strahilevitz*, 668 F.2d 1229, 1232, 212 USPQ 561, 563 (CCPA 1982).

ments in genetic engineering produce, at best, unpredictable results", quoting from *Ex parte Forman*, 230 USPQ 546, 547 (Bd.Pat.App. and Int. 1986).

The premise of the patent system is that an inventor, having taught the world something it didn't know, is encouraged to make the product available for public and commercial benefit, by governmental grant of the right to exclude others from practice of that which the inventor has disclosed. The boundary defining the excludable subject matter must be carefully set: it must protect the inventor, so that commercial development is encouraged; but the claims must be commensurate with the inventor's contribution. Thus the specification and claims must meet the requirements of 35 U.S.C. §112. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 23-24 (CCPA 1970).

As the science of biotechnology matures the need for special accommodation, such as the deposit of cell lines or microorganisms, may diminish; but there remains the body of law and practice on the need for sufficient disclosure, including experimental data when appropriate, that reasonably support the scope of the requested claims. That law relates to the sufficiency of the description of the claimed invention, and if not satisfied by deposit, must independently meet the requirements of Section 112.

Wands is not claiming a particular, specified IgM antibody. He is claiming all such monoclonal antibodies in assay for hepatitis B surface antigen, based on his teaching that such antibodies have uniformly reproducible high avidity, free of the known disadvantages of IgM antibodies such as tendency to precipitate or aggregate. It is incumbent upon Wands to provide reasonable support for the proposed breadth of his claims. I agree with the Commissioner that four exemplars shown to have the desired properties, out of the 143, do not provide adequate support.

Wands argues that the law should not be "harsher" where routine experiments take a long time. However, what Wands is requesting is that the law be less harsh. As illustrated in extensive precedent on the question of how much experimentation is "undue", each case must be determined on its own facts. *See, e.g., W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1557, 220 USPQ 303, 316 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 218 (CCPA 1976); *In re Cook*, 439 F.2d 730, 734-35, 169 USPQ 298, 302-03 (CCPA 1971).

The various criteria to be considered in determining whether undue experimentation is required are discussed in, for example, *Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971); *In re Rainer*, 347 F.2d 574, 146 USPQ 218 (CCPA 1965); *Ex parte Forman*, 230 USPQ at 547. Wands must provide sufficient data or authority to show that his results are reasonably predictable within the scope of the claimed generic invention, based on experiment and/or scientific theory. In my view he has not met this burden.

Patent and Trademark Office
Trademark Trial and Appeal Board

In re Johanna Farms Inc.

Serial No. 542,343

Decided June 30, 1988

JUDICIAL PRACTICE AND PROCEDURE

1. Procedure — Prior adjudication — In general (§410.1501)

Trademark Trial and Appeal Board's prior decision upholding examiner's refusal to register proposed mark "La Yogurt" does not preclude registration of mark pursuant to subsequent application, since applicant, by presenting survey evidence and consumer letters regarding issue of how purchasers perceive proposed mark, has demonstrated that instant factual situation is different from situation presented in prior proceeding.

TRADEMARKS AND UNFAIR TRADE PRACTICES

2. Types of marks — Non-descriptive — Particular marks (§327.0505)

Term "La Yogurt," with "yogurt" disclaimed, is registrable, since word "yogurt" is common English generic term rather than corruption or misspelling of French word for yogurt, since examining attorney failed to meet burden of showing clear evidence of generic use of mark as whole, and since evidence of record, including survey and consumer letters to applicant, demonstrates that primary significance of "La Yogurt" to majority of relevant public is that of brand name rather than generic term.

ARTHRITIS & RHEUMATISM Volume 36
Number 12, December 1993, pp. 1681–1690

TREATMENT OF RHEUMATOID ARTHRITIS WITH CHIMERIC MONOCLONAL ANTIBODIES TO TUMOR NECROSIS FACTOR α

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Objective. **To evaluate the safety and efficacy of a chimeric monoclonal antibody to tumor necrosis factor α (TNFα) in the treatment of patients with rheumatoid arthritis (RA).**

Methods. **Twenty patients with active RA were treated with 20 mg/kg of anti-TNFα in an open phase I/II trial lasting 8 weeks.**

Results. **The treatment was well tolerated, with no serious adverse events. Significant improvements were seen in the Ritchie Articular Index, which fell from a median of 28 at study entry to a median of 6 by week 6 ($P < 0.001$), the swollen joint count, which fell from 18 to 5 ($P < 0.001$) over the same period, and in the other major clinical assessments. Serum C-reactive protein levels fell from a median of 39.5 mg/liter at study entry to 8 mg/liter at week 6 ($P < 0.001$), and significant decreases were also seen in serum amyloid A and interleukin-6 levels.**

Conclusion. **Treatment with anti-TNFα was safe and well tolerated and resulted in significant clinical and laboratory improvements. These preliminary results support the hypothesis that TNFα is an important regulator in RA, and suggest that it may be a useful new therapeutic target in this disease.**

Despite optimal use of current antirheumatic therapy, the outcome for many patients with rheumatoid arthritis (RA) consists of pain, disability, and premature death (1–3). As a response to the need for more effective and less toxic treatment, and to an increase in our understanding of the pathogenic mechanisms in RA, several groups have used monoclonal antibodies as therapeutic agents in this disease (4–10). Such immunotherapy has been, in most cases, targeted specifically to the T cell, a strategy based on evidence that T cells are involved in the initiation and maintenance of RA (11).

Here, we outline an alternative immunotherapeutic strategy, which involves the use of monoclonal antibodies with specificity for a cytokine, tumor necrosis factor α (TNFα). This approach is based on a body of knowledge regarding the role of cytokines in general, and of TNFα in particular, in the inflammatory process in RA. The first clearly documented study demonstrated the presence of interleukin-1 (IL-1) in RA synovial fluid (12). Subsequently, we and others have reported the presence and local synthesis in

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Supported in part by grants from the Arthritis and Rheumatism Council of Great Britain. Dr. Elliott's work was supported by the Michael Mason Fellowship from the Arthritis Foundation of Australia, and by the DEV Starr Fellowship from the Royal Australasian College of Physicians.

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Submitted for publication May 28, 1993; accepted in revised form August 10, 1993.

Table 1. Demographic features of 20 patients with refractory rheumatoid arthritis

Patient	Age/sex	Disease duration (years)	Previous DMARDs*	Concomitant therapy†
1	48/F	7	SSZ, DP, GST, AUR, MTX, AZA, HCQ	Pred. 5 mg
2	63/F	7	SSZ, GST, DP	Para. 1–2 gm
3	59/M	3	AUR, HCQ, GST, MTX, SSZ	Pred. 10 mg, Indo. 225 mg
4	56/M	10	GST, DP, AZA, SSZ	Pred. 12.5 mg, Ibu. 2 gm, Para. 1–2 gm
5	28/F	3	GST, SSZ, DP, AZA	Pred. 8 mg, Para. 1–2 gm, Code. 16 mg
6	40/M	3	SSZ, HCQ, AUR	Nap. 1 gm
7	54/F	7	DP, GST, SSZ, AZA, MTX	Para. 1–2 gm, Code. 16–32 mg
8	23/F	11	HCQ, GST, SSZ, MTX, AZA	Pred. 7.5 mg, Dicl. 100 mg, Para. 1–2 gm, Dext. 100–200 mg
9	51/F	15	GST, HCQ, DP, MTX	Pred. 7.5 mg, Dicl. 125 mg, Para. 1–3 gm
10	47/F	12	SSZ, CYC, MTX	Ben. 4 gm
11	34/F	10	DP, SSZ, MTX	Pred. 10 mg, Para. 1.5 gm, Code. 30–90 mg
12	57/F	12	GST, MTX, DP, AUR	Asp. 1.2 gm
13	51/F	7	SSZ, AZA	Para. 1–4 gm
14	72/M	11	GST, DP, AZA, MTX	Pred. 5 mg, Para. 1–4 gm, Code. 16–64 mg
15	51/F	17	HCQ, DP, SSZ, MTX	Asp. 0.3 gm
16	62/F	16	GST, DP, SSZ, MTX, AZA	Para. 1–4 gm, Code. 16–64 mg
17	56/F	11	SSZ, DP, GST, MTX, HCQ, AZA	Pred. 7.5 mg, Eto. 600 mg, Para. 1–2 gm, Dext. 100–200 mg
18	48/F	14	GST, MTX, DP, SSZ, AUR, AZA	Pred. 7.5 mg, Indo. 100 mg, Para. 1–3 gm
19	42/F	3	SSZ, MTX	Fen. 450 mg, Ben. 6 gm, Code. 30 mg
20	47/M	20	GST, DP, SSZ, AZA	Pred. 10 mg, Para. 1–3 gm

* Disease-modifying antirheumatic drugs (DMARDs) were SSZ = sulfasalazine; DP = D-penicillamine; GST = gold sodium thiomalate; AUR = auranofin; MTX = methotrexate; AZA = azathioprine; HCQ = hydroxychloroquine; CYC = cyclophosphamide.
† Daily doses are shown. Pred. = prednisolone; Para. = paracetamol; Indo. = indomethacin; Ibu. = ibuprofen; Code. = codeine phosphate; Nap. = naprosyn; Dicl. = diclofenac; Dext. = dextropropoxyphene; Ben. = benorylate; Asp. = aspirin; Eto. = etodolac; Fen. = fenbufen.

rheumatoid synovial membrane of many cytokines, including IL-1 (13), TNFα (13,14), IL-6 (15), granulocyte-macrophage colony-stimulating factor (GM-CSF; 16), IL-8 (17), and transforming growth factor β (TGFβ) (18,19).

We have investigated the relationships between these cytokines in RA, using a synovial culture system in which dissociated rheumatoid synovial cells are allowed to spontaneously re-aggregate in vivo. Even in the absence of extrinsic stimulation, such cells express high levels of cytokines and HLA class II molecules (20). Using this system, we showed that production of bioactive IL-1 was abrogated by neutralizing antibodies to TNFα, but not by antibodies to TNFβ or by normal rabbit IgG (21). This occurred in rheumatoid, but not osteoarthritic, cultures and suggested to us that TNFα was of particular importance as a regulatory cytokine. Subsequent analysis reinforced this concept, with the demonstration that another proinflammatory cytokine, GM-CSF, was regulated in the synovial membrane by TNFα (22) and that TNFα receptor expression, necessary for transmitting TNFα signals, was up-regulated in rheumatoid synovium (23,24).

Two recent mouse studies provide further insight into the importance of TNFα in arthritis. Keffer et al (25) described a mouse transgenic for the human TNFα gene, which expressed high levels of human TNFα in vivo and which reproducibly developed arthritis beginning at 4 weeks of age. The disease in these animals could be prevented by administration of monoclonal antibodies to human TNFα. In separate experiments in our own laboratory, we showed that in the type II collagen arthritis model in the DBA/1 mouse, the hamster anti-murine TNF monoclonal antibody TN3.19.2 significantly ameliorated the inflammation and tissue destruction when administered before or after the onset of disease (26).

Based on these considerations, it was of interest to determine the effect of therapy with a chimeric (human IgG1, murine Fv) monoclonal antibody to human TNFα in patients with rheumatoid arthritis. We report here that anti-TNFα therapy was safe and well tolerated, and induced marked improvements in both clinical and laboratory disease measures. These findings are consistent with our postulate concerning the critical role of TNFα in the pathogenesis of RA (27,28), and suggest that TNFα may be a useful therapeutic target in this disease.

Table 2. Changes in clinical assessments following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Morning stiffness, minutes	Pain score, 0–10 cm	Ritchie index, 0–69	Swollen joint count, 0–28	Grip strength, 0–300 mm Hg		IDA, 1–4	Patient's assessment, no. grades improved, 0–3
					Left hand	Right hand		
Screen	135, 0–600	7.4, 4–9.7	23, 4–51	16, 4–28	84, 45–300	96, 57–300	3, 2.3–3.3	NA
0	180, 20–600	7.1, 2.7–9.7	28, 4–52	18, 3–27	77, 52–295	92, 50–293	3, 2–3.5	NA
1	20, 0–180 (<0.001†)	2.6, 0.6–7.8 (<0.001†)	13, 2–28 (<0.001; <0.002†)	13.5, 1–25 (>0.05)	122, 66–300 (>0.05)	133, 57–300 (>0.05)	2, 1.5–3.3 (<0.001†)	1, 1–3
2	15, 0–150 (<0.001†)	3.0, 0.3–6.4 (<0.001†)	13, 1–28 (<0.001†)	11.5, 1–22 (<0.003; <0.02†)	139, 75–300 (<0.03; >0.05†)	143, 59–300 (>0.05)	2, 1.5–3.2 (<0.001†)	1.5, 1–3
3	5, 0–150 (<0.001†)	2.2, 0.2–7.4 (<0.001†)	8, 0–22 (<0.001†)	6, 1–19 (<0.001; <0.002†)	113, 51–300 (>0.05)	142, 65–300 (>0.05)	2, 1.2–3.2 (<0.001†)	2, 1–2
4	15, 0–90 (<0.001†)	1.9, 0.1–5.6 (<0.001†)	10, 0–17 (<0.001†)	6, 0–21 (<0.001; <0.002†)	124, 79–300 (<0.02; >0.05†)	148, 64–300 (<0.03; >0.05†)	1.8, 1.3–2.7 (<0.001†)	2, 1–2
6	5, 0–90 (<0.001†)	1.9, 0.1–6.2 (<0.001†)	6, 0–18 (<0.001†)	5, 1–14 (<0.001†)	119, 68–300 (<0.04; >0.05†)	153, 62–300 (<0.05; >0.05†)	1.7, 1.3–2.8 (<0.001†)	2, 1–2
8	15, 0–60 (<0.001†)	2.1, 0.2–7.7 (<0.001†)	8, 1–28 (<0.001†)	7, 1–18 (<0.001†)	117, 69–300 (<0.03; >0.05†)	167, 53–300 (<0.03; >0.05†)	1.8, 1.5–2.8 (<0.001†)	2, 1–3

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. All *P* values versus week 0, by Mann-Whitney test. IDA = Index of Disease Activity; NA = not applicable.
† Adjusted for multiple statistical comparisons.

PATIENTS AND METHODS

Patient selection. Twenty patients were recruited, each of whom fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for the diagnosis of RA (29). The clinical characteristics of the patients are shown in Table 1. The study group comprised 15 females and 5 males, with a median age of 51 years (range 23–72), a median disease duration of 10.5 years (range 3–20), and a history of failed therapy with standard disease-modifying antirheumatic drugs (DMARDs) (median number of failed DMARDs 4, range 2–7).

Seventeen patients were seropositive at study entry or had been seropositive at some stage of their disease. All had erosions evident on radiographs of the hands or feet, and 3 had rheumatoid nodules. All patients had active disease at trial entry, as defined by an Index of Disease Activity (IDA) (30) of at least 1.75, together with at least 3 swollen joints, and were classified in anatomic and functional stage II or III (31). The pooled data for each of the clinical and laboratory indices of disease activity at the time of screening for the trial (up to 4 weeks prior to trial entry), and on the day of trial entry itself (week 0), are shown in Tables 2 and 3.

All DMARDs were discontinued at least 1 month prior to trial entry. Patients were allowed to continue taking a nonsteroidal antiinflammatory drug and/or prednisolone (≤12.5 mg/day) during the trial. The dosage of these agents was kept stable for 1 month prior to trial entry and during the course of the trial. No parenteral corticosteroids were allowed during these periods. Simple analgesics were allowed ad libitum.

Patients with other serious medical conditions were excluded from study. Specific exclusions were as follows: serum creatinine >150 μmoles/liter (normal 60–120), hemoglobin (Hgb) <90 gm/liter (normal 120–160 in females, and 135–175 in males), white blood cell (WBC) count <4 × 10^9/liter (normal 4–11 × 10^9/liter), platelet count <100 × 10^9/liter (normal 150–400 × 10^9/liter), and abnormal liver enzyme levels or active pathology noted on chest radiographs.

All patients gave their informed consent for the trial, and approval was granted by the local ethics committee.

Treatment protocol. cA2 is a chimeric human/mouse monoclonal anti-TNFα antibody, consisting of the constant regions of human (Hu)IgG1κ, coupled to the Fv region of a high-affinity neutralizing murine anti-HuTNFα antibody (A2). The antibody was produced by Centocor Inc., by continuous fermentation of a mouse myeloma cell line which had been transfected with cloned DNA coding for cA2, and was purified from culture supernatant by a series of steps involving column chromatography. The chimeric antibody retains specificity for natural and recombinant HuTNFα, and is of high affinity.

The antibody was stored at 4°C in 20-ml vials containing 5 mg of cA2 per milliliter of 0.01*M* phosphate buffered saline in 0.15*M* sodium chloride at a pH of 7.2 and was filtered through a 0.2-μm sterile filter before use. The appropriate amount of cA2 was then diluted to a total volume of 300 ml in sterile saline and administered intravenously via a 0.2-μm in-line filter over a period of 2 hours.

Patients were admitted to the hospital for 8–24 hours for each treatment, and were mobile except during infusions. The trial was of an open, uncontrolled design, with a comparison of 2 treatment schedules. Patients 1–5 and 11–20 received a total of 2 infusions, each consisting of 10 mg/kg of cA2, at entry to the study (week 0) and 14 days later (week 2). Patients 6–10 received a total of 4 infusions of 5 mg/kg at cA2, at entry and on days 4, 8, and 12. The total dose received by the 2 patient groups was therefore the same: 20 mg/kg.

Assessments. *Safety monitoring.* Vital signs were recorded every 15–30 minutes during infusions, and at intervals for up to 24 hours postinfusion. Patients were

Table 3. Changes in laboratory measures following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Hgb, gm/liter	WBC, $\times 10^9$/liter	Platelets, $\times 10^9$/liter	ESR, mm/hour	CRP, mg/liter	SAA, mg/ml	RF, inverse titer
Screen	117, 98–146	7.9, 3.9–15.2	352, 274–631	59, 18–87	42, 9–107	ND	ND
0	113, 97–144	9.0, 4.9–15.7	341, 228–710	55, 15–94	39.5, 5–107	245, 18–1,900	2,560, 160–10,240
1	114, 96–145 (>0.05)	8.5, 3.6–13.6 (>0.05)	351, 223–589 (>0.05)	26, 13–100 (>0.05)	5, 0–50 (<0.001†)	58, 0–330 (<0.001; <0.003†)	ND
2	112, 95–144 (>0.05)	8.2, 4.3–12.7 (>0.05)	296, 158–535 (<0.04; >0.05†)	27, 10–90 (<0.02; >0.05†)	5.5, 0–80 (<0.001; <0.003†)	80, 11–900 (<0.02; <0.04†)	ND
3	110, 89–151 (>0.05)	9.0, 3.7–14.4 (>0.05)	289, 190–546 (<0.03; >0.05†)	27, 12–86 (<0.04; >0.05†)	7, 0–78 (<0.001; <0.002†)	ND	ND
4	112, 91–148 (>0.05)	8.2, 4.7–13.9 (>0.05)	314, 186–565 (>0.05)	23, 10–87 (<0.04; >0.05†)	10, 0–91 (<0.004; <0.02†)	ND	ND
6	116, 91–159 (>0.05)	9.1, 2.9–13.9 (>0.05)	339, 207–589 (>0.05)	23, 12–78 (<0.03; >0.05†)	8, 0–59 (<0.001†)	ND	ND
8	114, 94–153 (>0.05)	7.6, 4.2–13.5 (>0.05)	339, 210–591 (>0.05)	30, 7–73 (>0.05)	6, 0–65 (<0.001†)	ND	480, 40–5,120 (>0.05)

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. For rheumatoid factor (RF), only those patients with week 0 titers ≥1:160 in the particle agglutination assay were included (n = 14). All *P* values versus week 0, by Mann-Whitney test. Normal ranges: hemoglobin (Hgb) 120–160 gm/liter in females and 135–175 gm/liter in males; white blood cell (WBC) count 4–11 × 10^9/liter; platelet count 150–400 × 10^9/liter; erythrocyte sedimentation rate (ESR) <15 mm/hour in females and <10 mm/hour in males; C-reactive protein (CRP) <10 mg/liter; serum amyloid A (SAA) <10 mg/ml. ND = not done.

questioned concerning possible adverse events before each infusion and at weeks 1, 2, 3, 4, 6, and 8 of the trial. A complete physical examination was performed at screening and at week 8. In addition, patients were monitored by standard laboratory tests including a complete blood cell count, and levels of C3 and C4 components of complement, IgG, IgM, and IgA, serum electrolytes, creatinine, urea, alkaline phosphatase, aspartate transaminase, and total bilirubin.

Sample times for these tests were between 0800 and 0900 hours (preinfusion) and 1200–1400 hours (24 hours postinfusion). Blood tests subsequent to day 1 were performed in the morning, usually between 0700 and 1200 hours. Urine analysis and culture were also performed at each assessment point.

Response assessment. The patients were assessed for response to cA2 at weeks 1, 2, 3, 4, 6, and 8 of the trial. The assessments were all made between 0700 and 1300 hours by the same observer (AL-F). The following clinical assessments were made: duration of morning stiffness (minutes), pain score (0–10 cm on a visual analog scale), Ritchie Articular Index (maximum score 69) (32), number of swollen joints (28 joint count) (validation described in ref. 33), grip strength (0–300 mm Hg, mean of 3 measurements per hand, by sphygmomanometer cuff), and an assessment of function (the Stanford Health Assessment Questionnaire [HAQ], modified for British patients [34]). In addition, the patients' global assessments of response were recorded using a 5-point scale (worse, no response, fair response, good response, excellent response).

Routine laboratory indicators of disease activity included complete blood cell counts, C-reactive protein (CRP) levels (by rate nephelometry), and the erythrocyte sedimentation rate (ESR; Westergren). Followup assessments were made at monthly intervals after the conclusion of the formal trial period, in order to assess the duration of response.

Analysis of improvement in individual patients was made using two separate indices. First, an IDA was calculated for each time point according to the method of Mallya and Mace (30), with input variables of morning stiffness, pain score, Ritchie Articular Index, grip strength, ESR, and Hgb. The second index calculated was that of Paulus et al (35), which uses input variables of morning stiffness, ESR, joint pain/tenderness, joint swelling, and patient's and physician's global assessments of disease severity.

To calculate the presence (or otherwise) of a response according to this index, two approximations were made to accommodate our data. The swollen joint count used by us (nongraded total of swollen joints of 28 joints assessed), which has been validated (33), was used in place of the more extensive graded count described by Paulus et al, and the patient's and physician's global assessments of *response* recorded by us were approximated to the global assessments of *disease activity* used by Paulus et al (35). In addition to determining response according to these published indices, we selected 6 disease activity assessments of interest (morning stiffness, pain score, Ritchie Articular Index, swollen joint count, ESR, and CRP) and calculated their mean percentage improvement. We have used this value to give an indication of the degree of improvement seen in responding patients.

Immunologic investigations. Rheumatoid factors were measured using the rheumatoid arthritis particle agglutination assay (RAPA) (FujiBerio Inc, Tokyo, Japan), in which titers of 1:160 or greater were considered significant. Rheumatoid factor isotypes were measured by enzyme-linked immunosorbent assay (ELISA) (Cambridge Life Sciences, Ely, UK). Addition of cA2, at concentrations of up to 200 μg/ml, to these assay systems did not alter the assay results (data not shown).

Antinuclear antibodies were detected by immunoflu-

orescence on HEp-2 cells (Biodiagnostics, Upton, UK), and antibodies to extractable nuclear antigens were measured by counterimmunoelectrophoresis with polyantigen extract (Biodiagnostics). Sera positive by immunofluorescence were also screened for antibodies to DNA by the Farr assay (Kodak Diagnostics, Amersham, UK). Anticardiolipin antibodies were measured by ELISA (Shield Diagnostics, Dundee, Scotland). Serum amyloid A (SAA) was measured by sandwich ELISA (Biosource International, Camarillo, CA). Antiglobulin responses to the infused chimeric antibody were measured by an in-house ELISA, using cA2 as a capture reagent.

Cytokine assays. Bioactive TNF was measured in sera using the WEHI 164 clone 13 cytotoxicity assay (36). Total IL-6 was measured in sera using a commercial immunoassay (Medgenix Diagnostics, Brussels, Belgium) and using a sandwich ELISA developed in-house, with monoclonal antibodies provided by Dr. F. di Padova (Basel, Switzerland). Microtiter plates were coated with monoclonal antibody LNI 314-14 at a concentration of 3 μg/ml for 18 hours at 4°C, and blocked with 3% bovine serum albumin in 0.1*M* phosphate buffered saline, pH 7.2. Undiluted sera or standards (recombinant HuIL-6, 0–8.1 μg/ml) were added to the wells in duplicate and incubated for 18 hours at 4°C. Bound IL-6 was detected by incubation with monoclonal antibody LNI 110-14 for 90 minutes at 37°C, followed by biotin-labeled goat anti-murine IgG2b for 90 minutes at 37°C (Southern Biotechnology, Birmingham, AL). The assay was developed using streptavidin–alkaline phosphatase (Southern Biotechnology) and *p*-nitrophenyl phosphate as a substrate, and the optical density read at 405 nm.

Statistical analysis. Data for week 0 versus subsequent time points were compared for each assessment using the Mann-Whitney test. For comparison of rheumatoid factor titers (by RAPA), the data were expressed as dilutions before applying the test.

This was an exploratory study, in which prejudgments about the optimal times for assessment were not possible. Although it has not been common practice to adjust for multiple statistical comparisons in such studies (4–10), a conservative statistical approach would require adjustment of *P* values to take into account analysis at several time points. The *P* values have therefore been presented in two forms: unadjusted, and after making allowance for analysis at multiple time points by use of the Bonferroni adjustment. Where *P* values remained <0.001 after adjustment, a single value only is given. A *P* value of <0.05 is considered significant.

RESULTS

Safety of cA2. The administration of cA2 was exceptionally well tolerated, with no headache, fever, hemodynamic disturbance, allergy, or other acute manifestation. No serious adverse events were recorded during the 8-week trial. Two minor infective episodes were recorded, each "possibly related" to cA2: patient 15 presented at week 2 with clinical features of bronchitis. Sputum culture grew only normal commensals. She had a history of smoking and of a similar illness 3 years previously. The illness responded promptly to treatment with amoxicillin, but her second cA2 infusion was withheld and the data for this patient are therefore not analyzed beyond week 2. Patient 18 showed significant bacteriuria on routine culture at week 6 ($>10^5$/ml; lactose-fermenting coliform), but was asymptomatic. This condition also responded promptly to amoxicillin.

Routine analysis of blood samples showed no consistent adverse changes in hematologic parameters, renal function, liver function, or levels of C3, C4, or immunoglobulins during the 8 weeks of the trial. Four minor, isolated, and potentially adverse laboratory disturbances were recorded. Patient 2 experienced a transient rise in blood urea levels, from 5.7 mmoles/liter to 9.2 mmoles/liter (normal 2.5–7), with no change in serum creatinine. This change was associated with the temporary use of a diuretic, which had been prescribed for a non-rheumatologic disorder. The value normalized within 1 week and was classified as "probably not related" to cA2.

Patient 6 experienced a transient fall in the peripheral blood lymphocyte count, from 1.6×10^9/liter to 0.8×10^9/liter (normal 1.0–4.8). This abnormality was not seen at the next sample point (2 weeks later), was not associated with any clinical manifestations, and was classified as "possibly related" to cA2. Patients 10 and 18 developed elevated titers of anti-DNA antibodies at weeks 6 and 8 of the trial. Elevated anticardiolipin antibodies were also detected in patient 10. Both patients had a preexisting positive antinuclear antibody titer, and patient 10 had a history of borderline lymphocytopenia and high serum IgM. There were no clinical features of systemic lupus erythematosus, and the laboratory changes were judged "probably related" to cA2.

Efficacy of cA2. The pattern of response for each of the clinical assessments of disease activity and the derived IDA are shown in Table 2. All clinical assessments showed improvement following treatment with cA2, with maximal responses from week 3. Duration of morning stiffness decreased from a median of 180 minutes at study entry (week 0) to 5 minutes at week 6 ($P < 0.001$ by Mann-Whitney test, adjusted), representing a 97% improvement. The pain score decreased from 7.1 to 1.9 over the same period ($P < 0.001$, adjusted), representing an improvement of 73%. Similarly, the Ritchie Articular Index improved from 28 to 6 at week 6 ($P < 0.001$, adjusted; 79% improvement), and the swollen joint count decreased from 18

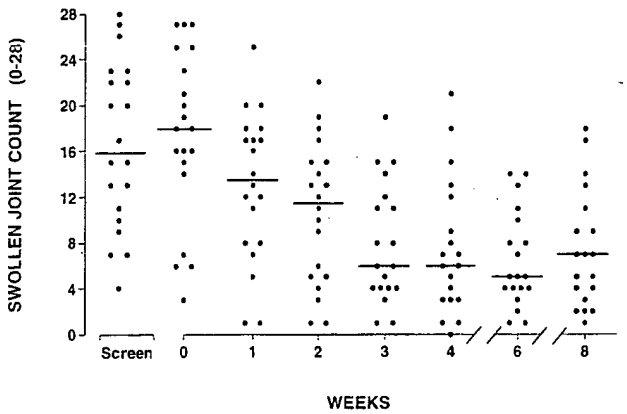

Figure 1. Swollen joint counts (maximum 28), as recorded by a single observer, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P > 0.05$ at week 1, $P < 0.02$ at week 2, $P < 0.002$ at weeks 3 and 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

to 5 ($P < 0.001$, adjusted; 72% improvement). The individual swollen joint counts for all time points are shown in Figure 1.

Grip strength also improved; the median grip strength rose from 77 mm Hg (left) and 92 mm Hg (right) at week 0 to 119 (left) and 153 (right) at week 6 ($P < 0.04$ and $P < 0.05$, left and right hands, respectively; $P > 0.05$ both hands, adjusted for multiple comparisons). The IDA has a range of 1 (normal) to 4 (severe disease activity). The IDA showed a decrease from a median of 3 at study entry to 1.7 at week 6 ($P < 0.001$, adjusted). Patients were asked to grade their responses to cA2 using a 5-point scale. No patient recorded a response of "worse" or "no change" at any point in the trial. "Fair," "good," and "excellent" responses were classified as improvements of 1, 2, and 3 grades, respectively. At week 6, there was a median of 2 grades of improvement (Table 2).

We also measured changes in the patients' functional capacity, using the HAQ, as modified for British patients (range 0–3). The median (range) HAQ score improved from 2 (0.9–3) at study entry to 1.1 (0–2.6) by week 6 ($P < 0.001$ and $P < 0.002$ adjusted).

The changes in the laboratory values which reflect disease activity are shown in Table 3. The most rapid and impressive changes were seen in serum CRP levels, which fell from a median of 39.5 mg/liter at week 0 (normal <10) to 8 mg/liter by week 6 of the trial ($P < 0.001$, adjusted), representing an improvement of 80%. Of the 19 patients with elevated CRP at study entry, 17 showed decreases to the normal range at some point during the trial. The improvement in CRP was maintained in most patients over the assessment period (Table 3 and Figure 2); the exceptions with high values at 4 and 6 weeks tended to be those with the highest starting values (data not shown).

The ESR also showed improvement, with a fall from 55 mm/hour at study entry (normal <10 in males and <15 in females) to 23 mm/hour at week 6 ($P < 0.03$ and $P > 0.05$ adjusted; 58% improvement). SAA levels were elevated in all patients at trial entry, and fell from a median of 245 mg/ml (normal <10) to 58 mg/ml at week 1 ($P < 0.003$ adjusted; 76% improvement) and to 80 mg/ml at week 2 ($P < 0.04$, adjusted). No significant changes were seen in Hgb level, WBC count, or platelet count at week 6, although the platelet count did improve at weeks 2 and 3 compared with trial entry (Table 3).

The response data were also analyzed for each patient individually (not shown). The majority of patients had their best overall responses at week 6, at which time 13 assessed their responses as "good" while 6 assessed their responses as "fair." Eighteen of the 19 patients who completed the treatment schedule achieved an improvement in the IDA of 0.5 or greater at week 6, and 10 achieved an improvement of 1.0 or greater. All patients achieved a response at week 6

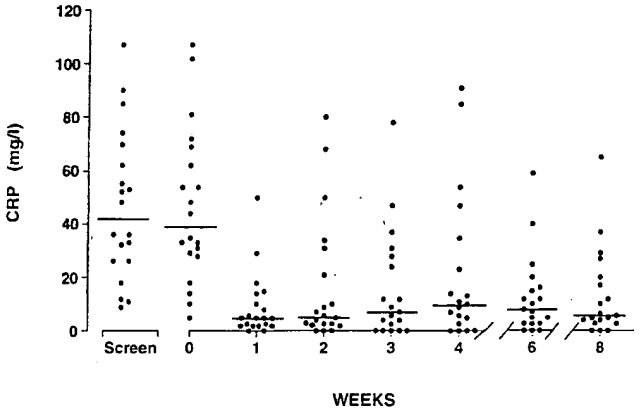

Figure 2. Serum C-reactive protein (CRP) levels (normal 0–10 mg/liter), as measured by nephelometry, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P < 0.001$ at week 1, $P < 0.003$ at week 2, $P < 0.002$ at week 3, $P < 0.02$ at week 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

according to the index described by Paulus et al (35). At week 6, all patients showed a mean improvement of 30% or greater in the 6 selected measures of disease activity (see Patients and Methods), with 18 of the 19 patients showing a mean improvement of 50% or greater (data not shown).

Although the study was primarily designed to assess the short-term effects of cA2 treatment, followup clinical and laboratory data are available for those patients followed for sufficient time (n = 12). The duration of response in these patients, defined as the duration of a 30% (or greater) mean improvement in the 6 selected disease activity measures, was variable, ranging from 8 weeks to 25 weeks (median 14) (data not shown).

Comparison of the clinical and laboratory data for patients treated with 2 infusions of cA2 (each at 10 mg/kg) versus those treated with 4 infusions (each at 5 mg/kg) showed no significant differences in the rapidity or extent of response (data not shown).

Immunologic investigations and cytokines. Measurement of rheumatoid factor by RAPA showed 14 patients with significant titers (≥1:160) at trial entry. Of these, 6 patients showed a decrease of at least 2 titers on treatment with cA2, while the remaining patients showed a change of 1 titer or less. No patient showed a significant increase in rheumatoid factor titer during the trial (data not shown). The median titer in the 11 patients decreased from 1:2,560 at entry to 1:480 by week 8 ($P > 0.05$) (Table 3). Specific rheumatoid factor isotypes were measured by ELISA, and showed decreases in the 6 patients whose RAPA had declined significantly, as well as in some other patients (data not shown). Median values for the 3 isotypes in the 14 patients seropositive at trial entry were 119, 102, and 62 IU/ml (IgM, IgG, and IgA isotypes, respectively) and at week 8 were 81, 64, and 46 IU/ml ($P > 0.05$).

We tested sera from patients 1–9 for the presence of bioactive TNF, using the WEHI 164 clone 13 cytotoxicity assay (36). In 8 patients, serum samples spanning the entire trial period were tested; while for patient 9, only 3 samples (1 pretrial, 1 intermediate, and the last available sample) were tested. The levels of bioactive TNF were below the limit of sensitivity of the assay in the presence of human serum (1 pg/ml) (data not shown).

Since production of CRP and SAA are thought to be regulated in large part by IL-6, we also measured serum levels of this cytokine, using 2 different assays which measure total IL-6. In the Medgenix assay, IL-6 was significantly elevated in 17 of the 20 patients at study entry. In this group, levels fell from 60 pg/ml (range 18–500) to 40 pg/ml (range 0–230) at week 1 ($P > 0.05$) and to 32 pg/ml (range 0–210) at week 2 ($P < 0.005$ and $P < 0.01$, adjusted). These results were supported by measurement of serum IL-6 in the first 16 patients in a separate ELISA developed in-house. IL-6 was detectable in 11 of these samples, with median (range) levels falling from 210 pg/ml (25–900) at entry to 32 pg/ml (0–1,700) at week 1 ($P < 0.02$ and $P < 0.04$, adjusted) and to 44 pg/ml (0–240) at week 2 ($P < 0.02$ and $P < 0.03$, adjusted).

We tested sera from patients 1–10 for the presence of antiglobulin responses to the infused chimeric antibody, but none were detected (data not shown). In many patients, however, cA2 was still detectable in serum samples taken at week 8 (data not shown) and this may have interfered with the ELISA.

DISCUSSION

This is the first report describing the administration of anti-TNFα antibodies for treatment of human autoimmune disease. Many cytokines are produced in rheumatoid synovium, but we chose to specifically target TNFα because of mounting evidence that it was a major molecular regulator in RA (21,22,26–28). The study results presented here support that view and allow 3 important conclusions to be drawn.

First, treatment with cA2 was safe and the infusion procedure was well tolerated. Although fever, headache, chills, and hemodynamic disturbance have all been reported following treatment with anti-CD4 or anti-CDw52 in RA (6,10), such features were absent in our patients. Also notable was the absence of any allergic event despite repeated treatment with the chimeric antibody, although the interval between initial and repeat infusions may have been too short to allow maximal expression of any antiglobulin response. The continuing presence of circulating cA2 at the conclusion of the trial may have precluded detection of antiglobulin responses, but also indicated that any such responses were likely to be of low titer and/or affinity. Although we recorded 2 episodes of infection among the study group, these were minor and the clinical courses were unremarkable. TNFα has been implicated in the control of *Listeria* and other infections in mice (37), but our limited experience does not suggest an increased risk of infection after TNFα blockade in humans.

The second conclusion concerns the clinical

efficacy of cA2. The patients we treated had longstanding, erosive, and for the most part, seropositive disease, and therapy with several standard DMARDs had failed. Despite this, the major clinical assessments of disease activity and outcome (morning stiffness, pain score, Ritchie articular index, swollen joint count, and HAQ score) showed statistically significant improvement, even after adjustment for multiple comparisons. All patients graded their response as at least "fair," with the majority grading it as "good." In addition, all achieved a response according to the criteria of Paulus et al and showed a mean improvement of at least 30% in 6 selected disease activity measures. The design of the trial does not allow these results to be attributed to the action of cA2 alone. However, the extent of the clinical improvements, their consistency throughout the study group, and the parallel changes in laboratory indices of disease activity (see below) are encouraging.

The improvements in clinical assessments following treatment with cA2 appear to be at least as good as those reported following treatment of similar patients with antileukocyte antibodies (6,10), although firm conclusions concerning each of these agents will require controlled, blinded studies. The two therapeutic approaches may already be distinguished, however, by their effects on the acute-phase response, since in several studies of antileukocyte antibodies, no consistent improvements in CRP or ESR were seen (4–6,8,10). In contrast, treatment with cA2 resulted in significant decreases in serum CRP and SAA values, with normalization of values in many patients. The changes were rapid and marked, and in the case of CRP, sustained for the duration of the study (Table 3). The decreases in ESR were less marked, achieving statistical significance only when unadjusted for the number of comparisons (Table 3).

These results are consistent with current concepts that implicate TNFα in the regulation of hepatic acute-phase protein synthesis, either directly, or by control of other, secondary, cytokines such as IL-6 (38,39). To investigate the mechanism of control of the acute-phase response in our patients, we measured serum TNFα and IL-6 before and after cA2 treatment. Bioactive TNFα was not detectable in sera obtained at baseline or subsequently. In view of previous reports of variability between different immunoassays in the measurement of cytokines in biologic fluids (40), we used 2 different assays for IL-6, and both demonstrated significant decreases in serum IL-6 levels by week 2. These findings support the other objective laboratory changes induced by cA2, and provide in vivo evidence that TNFα may be a regulatory cytokine for IL-6 in this disease. Among the other laboratory tests performed, levels of rheumatoid factors fell significantly in 6 patients.

The mechanism of action of cA2 leading to the clinical responses outlined above was not established in this study. Neutralization of TNFα may have a number of beneficial consequences, including a reduction in the local release of cytokines such as IL-6 and other inflammatory mediators, and modulation of synovial endothelial/leukocyte interactions. cA2 may also bind directly to synovial inflammatory cells expressing membrane TNFα, with subsequent in situ cell lysis. Further studies should establish which actions of cA2 may be clinically important.

The results obtained in this small series have important implications, both scientifically and clinically. At the scientific level, the ability of the neutralizing antibody, cA2, to reduce acute-phase protein synthesis, reduce the production of other cytokines such as IL-6, and significantly improve the clinical state demonstrates that it is possible to interfere with the cytokine network in a useful manner without untoward effects. Due to the many functions and overlapping effects of cytokines such as IL-1 and TNFα, and the fact that cytokines induce the production of other cytokines and of themselves, there had been some pessimism as to whether targeting a single cytokine in vivo would have any beneficial effect (41,42). This view is clearly refuted. On the clinical side, the results of short-term treatment with cA2 are encouraging, and suggest that TNFα may be a useful new therapeutic target in RA.

ACKNOWLEDGMENTS

We thank Drs. R. McCloskey, M. Sanders, U. Nassander, C. Wortel, D. Zelinger, and R. Lorijn of Centocor Inc. for their assistance with trial management, Dr. F. di Padova for the kind gift of anti–IL-6 monoclonal antibodies, Drs. J. Kirwan, H. Bird, and P. Schur for reviewing the manuscript, and the following physicians for the referral of patients included in this study: S. Allard, M. Corbett, C. Erhardt, A. Keat, C. Higgins, C. Mackworth-Young, G. Room, A. So, and P. Venables. We also thank Ms A. Hogg, Ms S. Tyler, and Ms G. Harris for excellent technical assistance and Dr. C. Plater-Zyberk for help in videotaping patient interviews.

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Inhibition of immunoreactive tumor necrosis factor-alpha by a chimeric antibody in patients infected with human immunodeficiency virus type 1.

J Infect Dis. 1996 Jul;174(1):63-8. Unique Identifier : AIDSLINE MED/96261994

Walker RE; Spooner KM; Kelly G; McCloskey RV; Woody JN; Falloon J; Baseler M; Piscitelli SC; Davey RT Jr; Polis MA; Kovacs JA; Masur H; Lane HC; National Institute of Allergy and Infectious Diseases, Critical; Care Medicine Department, National Institutes of Health,; Bethesda, MD 20892, USA.

Abstract: Tumor necrosis factor-alpha (TNF-alpha), a proinflammatory cytokine known to stimulate human immunodeficiency virus type 1 (HIV-1) replication, has been implicated in the pathogenesis of HIV-1 infection. Inhibition of TNF-alpha by a chimeric humanized monoclonal antibody, cA2, was investigated in 6 HIV-1-infected patients with CD4 cell counts < 200/mm3. Two consecutive infusions of 10 mg/kg 14 days apart were well tolerated, and a prolonged serum half-life for cA2 (mean, 257 +/- 70 h) was demonstrated. Serum immunoreactive TNF-alpha concentrations fell from a mean prestudy value of 6.4 pg/mL (range, 4.2-7.9) to 1.1 pg/mL (range, 0.5-2.2) 24 h after the first infusion and returned to baseline within 7-14 days. A similar response was seen after the second infusion. No consistent changes in CD4 cell counts or plasma HIV RNA levels were observed over 42 days. Future studies evaluating the therapeutic utility of long-term TNF-alpha suppression using anti-TNF-alpha antibodies are feasible and warranted.

Keywords: Acquired Immunodeficiency Syndrome/BLOOD/*IMMUNOLOGY Adult Animal Antibodies/*THERAPEUTIC USE Antibodies, Monoclonal/*THERAPEUTIC USE Chimeric Proteins/PHARMACOKINETICS/*THERAPEUTIC USE Female Human *HIV-1 Male Mice Recombinant Proteins/PHARMACOKINETICS/THERAPEUTIC USE Tumor Necrosis Factor/*ANTAGONISTS & INHIB/IMMUNOLOGY JOURNAL ARTICLE
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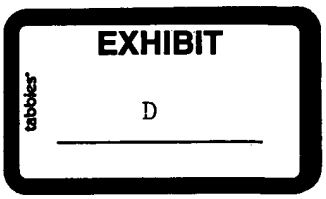

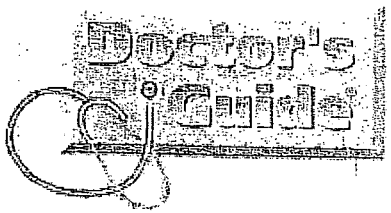

Medical & Other News

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Title: **New Monoclonal Antibody Effective Treatment For Crohn's Disease Therapy**
URL: http://www.pslgroup.com/dg/2802E.htm
Doctor's Guide
May 13, 1997

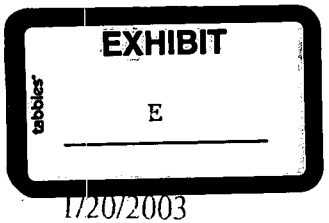
WASHINGTON, and MALVERN, Pa., May 13, 1997 -- Statistically significant results were released yesterday from two controlled clinical studies testing cA2(TM) (infliximab), a monoclonal antibody, in the treatment of Crohn's disease, a chronic disorder characterized by inflammation of the gastrointestinal tract. Data from both trials show that treatment with cA2 can have a beneficial effect on both the severity and number of symptoms associated with Crohn's disease.

"This kind of clinical response in Crohn's disease is unprecedented," said Stephan Targan, M.D., principal investigator and Director of the Inflammatory Bowel Disease Center at Cedars-Sinai Medical Center in Los Angeles, "and provides compelling evidence of the potential of cA2 in the treatment of Crohn's disease."

The results of these trials, which were conducted in 18 centers in North America and Europe, were announced today during Digestive Disease Week in Washington, DC. Digestive Disease Week is sponsored by the American Association for the Study of Liver Diseases, the American Gastroenterological Association, the American Society for Gastrointestinal Endoscopy and The Society for Surgery of the Alimentary Tract.

Last year, during Digestive Disease Week, Centocor released data showing a statistically significant improvement in disease activity following a single infusion of cA2. In the initial study, 65 percent of patients treated with cA2 achieved a clinical response and 33 percent of patients went into remission within four weeks of the start of treatment.

In the extension phase of this study, known as T16, which is being reported today, additional cA2 treatments were demonstrated to maintain Crohn's disease patients in clinical remission as measured by the CDAI, the Crohn's disease activity index.



In the initial phase of the T16 trial, the median CDAI of treated patients dropped from 312 to 125 eight weeks after a single cA2 infusion. Following four additional infusions, given eight weeks apart in the most recent phase of the T16 trial, cA2 maintained the CDAI reduction, with median CDAI eight weeks following the final treatment at 117 (CDAI<150 constitutes disease remission).

Data from the second trial, named T20, indicate that cA2 may be a valuable treatment for enterocutaneous fistulae, a painful, debilitating complication of Crohn's disease in which extensions occur between the bowel and the skin, mostly in the perianal area, causing drainage of mucous and/or fecal material. In this trial, approximately two-thirds of participants experienced closure of at least 50 percent of their fistulae.

In both clinical trials, onset of cA2 clinical benefit was rapid with the vast majority of responders achieving response within two weeks. In addition, cA2 was generally well tolerated in these two trials. "We have been following these studies with great interest," said Richard P. MacDermott, M. D., Immediate Past Chairperson, National Scientific Advisory Committee, Crohn's & Colitis Foundation of America (CCFA). "The results are very encouraging. It is possible that an important new therapy for Crohn's disease may be on the horizon."

In the T16 study, 73 patients who showed a clinical response eight weeks after their initial infusion of cA2 were re-randomized at week 12 to further treatment with cA2 or placebo, and infused every eight weeks for a total of four additional infusions. Those patients re-randomized to cA2 continued to experience an improvement in symptoms from baseline assessment and the percentage of patients achieving clinical remission was maintained at approximately 60 percent during the re-treatment period.

Those patients who responded to their initial infusion of cA2 and then received placebo in the re-treatment phase of the study, experienced a gradual decline in clinical effect over time. However, 19 percent of the placebo group were still in remission 48 weeks after their initial cA2 infusion.

The second study, T20, was conducted with 94 patients with draining enterocutaneous fistulae. Following a series of three cA2 infusions given two and four weeks apart, two-thirds of patients experienced closure of at least 50 percent of their fistulas (P=0.002). These patients had previously failed to respond adequately to treatment with combinations of corticosteroids, methotrexate, 6-MP/azathioprine, aminosalicylates, or antibiotics. These underlying therapies were given in conjunction with the cA2 infusions in this study. "cA2 is the first drug to ever demonstrate statistical significance in a controlled trial to close fistulas," according to Daniel Present, M.D., principal investigator and Clinical Professor of Medicine at Mount Sinai.

cA2, a monoclonal antibody, is the first of a revolutionary class of agents being studied for Crohn's disease. It is a well-tolerated, highly selective treatment that blocks activity of a key inflammatory mediator called tumor necrosis factor or TNF. cA2 is also being studied for treatment of rheumatoid arthritis.

Centocor is a biotechnology company whose mission is to develop and commercialize novel therapeutic and diagnostic products and services that solve critical needs in human health care. The company concentrates on research and development, manufacturing and market development, with a primary technology focus on monoclonal antibodies and DNA-based products.

More information about the company and cA2 can be found on Centocor's home page located at the following address. For more information about Crohn's disease or ulcerative colitis, a related disorder, contact the Crohn's & Colitis Foundation of America, at 1-800-343-3637 (website: http://www.ccfa.org).

Return to News Story Page

-continued

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATCGGACGTGGACGTGCAGA 20

What is claimed is:

1. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 5.

2. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim 1, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

3. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

4. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim 3, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

5. A chimeric antibody, comprising two light chains and two heavy chains, each of said chains comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said variable region capable of binding an epitope of human tumor necrosis factor hTNFα, wherein said light chains comprise variable regions comprising SEQ ID NO: 3 and said heavy chains comprise variable regions comprising SEQ ID NO: 5.

6. A chimeric antibody according to claim 5, wherein the human immunoglobulin constant region is an IgG1.

7. A chimeric antibody comprising at least part of a human IgG1 constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

8. A polypeptide comprising the amino acid sequence of SEQ ID NO: 3, wherein said polypeptide binds to hTNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

9. A polypeptide comprising the amino acid sequence of SEQ ID NO: 5, wherein said polypeptide binds to hTNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

* * * * *